C:\NrPortbl\imanage\CWISE\707740_1.DOC
DES/CMW/atc
May 24, 2007

PATENT APPLICATION
Docket No.: 3518.1015-000

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appellants: Thomas M. DiMauro, Mohamed Attawia, Hassan Serhan, Martin A. Reynolds, Melissa Grace, Sudhakar Kadiyala, David Urbahns, Scott Bruder, Gregory Collins, Laura J. Brown, Jeff Geesin, Pamela L. Plouhar, Catherine Smith and John Siekierka

Application No.: 10/630,227 Group: 1647

Filed: July 30, 2003 Examiner: Shulamith H. Shafer

Confirmation No.: 8291

For: TRANS-CAPSULAR ADMINISTRATION OF HIGH SPECIFICITY CYTOKINE INHIBITORS INTO ORTHOPEDIC JOINTS

CERTIFICATE OF MAILING OR TRANSMISSION

I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as First Class Mail in an envelope addressed to Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, or is being facsimile transmitted to the United States Patent and Trademark Office on:

05/30/07 Kelley A. Farr
Date Signature

Kelley A. Farr
Typed or printed name of person signing certificate

06/04/2007 HBLANCO 00000008 080380 10630227
01 FC:1402 500.00 DA

APPEAL BRIEF

Mail Stop Appeal Brief Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

This Appeal Brief is submitted pursuant to the Notice of Appeal received in the U.S. Patent and Trademark Office on November 30, 2006, and in support of the appeal from the rejections set forth in the Office Action mailed on June 27, 2006 and in the Notice of Panel Decision from the Pre-Appeal Brief Review mailed January 25, 2007. The Examiner has twice rejected Claims 1, 2, 34, 36-43, 45-51, 53-58, 60-61 and 63-65. The authorization for charging the fee to Deposit Account No. 08-0380 for filing a brief in support of an appeal is enclosed. A Petition for Extension of Time and the authorization for charging the appropriate fee to Deposit Account No. 08-0380 are being filed concurrently.

I. REAL PARTY IN INTEREST

The real party in interest is DePuy Spine, Inc., a corporation existing under the laws of the State of Ohio, and having a usual place of business at 325 Paramount Drive, Raynham, MA 02767. DePuy Spine, Inc., is the Assignee of the entire right, title and interest in the subject application, by virtue of an Assignment recorded on at Reel 014950, Frames 0123-0150 and at Reel 015160, Frames 0123-0127.

II. RELATED APPEALS AND INTERFERENCES

Appellants, the undersigned Attorney and the Assignee are not aware of any related appeals or interferences which will directly affect or be directly affected by or have a bearing on the Board's decision in the pending appeal.

III. STATUS OF CLAIMS

A listing of appealed claims appears in the Claims Appendix of this Brief. Claims 1, 2, 34, 36-43, 45-51, 53-58, 60-65 and 89-92 are pending. Claims 14, 44 and 59 were canceled. Claims 3-13, 15-33, 35, 52 and 66-88 were withdrawn.

Claims 1, 2, 34, 36-43, 45-51, 53-58, 60-61 and 63-65 have been twice rejected. Claims 84-92 are pending but have not been twice rejected. Claim 62 was incorrectly treated as withdrawn in the first Office Action and was rejected in the second Office Action. Therefore, it is pending but has not been twice rejected. Thus, the claims under appeal are as follows: Claims 1, 2, 34, 36-43, 45-51, 53-58 and 60-61 and 63-65.

IV. STATUS OF AMENDMENTS

In an Amendment filed on April 4, 2006, Claims 1, 34, 38, 48, 51, 55, 63 and 65 were amended and Claims 84-92 were added. No amendments have been filed subsequent to the Office Action mailed June 27, 2006.

V. SUMMARY OF CLAIMED SUBJECT MATTER

Appellants' invention is directed to a method of treating an inflamed orthopedic joint comprising trans-capsularly administering into the joint space an inhibitor of TNF-α synthesis. As taught in Appellants' specification, direct administration of the inhibitor of TNF-α synthesis trans-capsularly is advantageous over systemic treatment. Such advantages include, for example, arresting the inflammation process commencing within the joint and the degeneration of the

hyaline cartilage, preventing intracapsular nerve irritation and pain, increasing the half life of the inhibitor of TNF-α synthesis in the capsule, reducing unwanted side effects and permitting combination with other therapeutic agents without reducing their effectiveness (see the specification, for example at page 7, line 19 to page 9, line 25).

There is one independent claim, Claim 1. Appellants' invention is a method of treating an inflamed orthopedic joint, said joint comprising i) opposing hyaline cartilage articular surfaces, ii) a peripheral collagenous capsule defining a central joint space and iii) synovial fluid contained within the joint space, comprising trans-capsularly administering into the joint space a formulation comprising an effective amount of an inhibitor of TNF-α synthesis such that the inflamed orthopedic joint is treated (Claim 1). In one particular aspect, the orthopedic joint is a knee joint (Claim 2). (see the specification, for example at page 11, line 23 to page 12, line 14).

In one particular aspect (Claim 51), the inhibitor of TNF-α synthesis therapeutically inhibits the production of a cytokine (see the specification, for example at page 14, lines 17-18). In another particular aspect (Claim 34), the administered formulation further comprises at least one growth factor (see the specification, for example at page 32, line 11 to page 33, line 2). In another particular aspect (Claim 49), the administered formulation further comprises a growth factor present in an amount effective to repair joint tissue. In another particular aspect (Claim 50), the growth factor is provided by platelet concentrate (see the specification, for example, at page 32,line 1 to page 33, line 10). In another particular aspect (Claim 54), the administered formulation includes a viscosupplement (see the specification, for example at page 23, line 22 to page 24, line 5).

In another particular aspect (Claim 37), the formulation is administered in an amount of less than 1 cc (see the specification, for example at page 22, line 28 to page 23, line 5). In another particular aspect (Claim 38), the inhibitor of TNF-α synthesis is present in the formulation in an amount of at least 100 mg/ml (see the specification, for example at page 23, lines 15-16). In another particular aspect (Claim 48), the inhibitor of TNF-α synthesis is present in the formulation in a maximum amount of 0.5 mg (see the specification, for example at page 22, line 10 to page 23, line 21).

In another particular aspect (Claim 39), the administered formulation further comprises a sustained release device. In another particular aspect (Claim 40), the sustained delivery device comprises a hydrogel. In another particular aspect (Claim 41), the sustained delivery device

provides controlled release. In another particular aspect (Claim 42), the sustained delivery device provides continuous release. In another particular aspect (Claim 43), the sustained delivery device provides intermittent release. In another particular aspect (Claim 45), the sustained delivery device comprises microspheres having a plurality of degradation rates. In another particular aspect (Claim 46), the sustained delivery device comprises an inflammatory-responsive delivery system (see the specification, for example at page 24, line 21 to page 25, line 17).

In another particular aspect (Claim 63), the inhibitor of TNF-α synthesis is predominantly released from the formulation by diffusion through a sustained delivery device. In another particular aspect (Claim 64), the sustained delivery device is a polymer. In another particular aspect (Claim 65), the inhibitor of TNF-α synthesis is predominantly released from the formulation by biodegradation of a sustained delivery device (see the specification, for example at page 24, line 21 to page 25, line 17).

In another particular aspect (Claim 47), the formulation is provided closely adjacent to the outer wall of the capsule (see the specification, for example at page 13, lines 1-2). In another particular aspect (Claim 60), the formulation is provided in a patch attached to an outer wall of the capsule (see the specification, for example at page 12, lines 17-29). In another particular aspect (Claim 61), the administration comprises providing the formulation in a depot at a location closely adjacent an outer wall of the capsule (see the specification, for example at page 13, lines 1-2). In another particular aspect (Claim 36), the administered formulation further comprises a liposomal delivery system (see the specification, for example at page 25, line 21 to page 26, line 12).

In another particular aspect (Claim 53), the formulation is injected into the synovial fluid (see the specification, for example at page 29, lines 13-17). In another particular aspect (Claim 55), a portion of the synovial fluid is removed prior to administration of the inhibitor of TNF-α synthesis (see the specification, for example at page 29, lines 23-25). In another particular aspect (Claim 56), the formulation is administered through a needle (see the specification, for example at page 29, lines 18-22). In another particular aspect (Claim 57), the formulation is administered through a drug pump (see the specification, for example at page 30, line 4). In another particular aspect (Claim 58), the formulation is administered in a volume of between 0.03 ml and 0.3 ml. (see the specification, for example at page 29, lines 5-11).

VI. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

A. Whether Claims 1, 2, 34, 37, 47, 49, 51, 54 and 56 are properly rejected under 35 U.S.C. § 103(a) as being obvious over Lehman *et al.*, *The Journal of Pediatrics, 140*:125-127 (2002) in view of Dunn (EP 1 153 606).

B. Whether Claims 36, 39-43, 45, 58, 60, 61, 63-65 are properly rejected under 35 U.S.C. § 103(a) as being obvious over Lehman *et al.*, *The Journal of Pediatrics, 140*:125-127 (2002) in view of Pike *et al.* (US Publication No. 20030134792).

C. Whether Claim 50 is properly rejected under 35 U.S.C. § 103(a) as being obvious over Lehman *et al.*, *The Journal of Pediatrics, 140*:125-127 (2002) in view of Pike *et al.* (US Publication No. 20030134792) and Molloy *et al. Sports Med., 33*:381-394 (2003).

D. Whether Claims 1, 53 and 57 are properly rejected under 35 U.S.C. § 103(a) as being obvious over Lehman *et al.*, *The Journal of Pediatrics, 140*:125-127 (2002) in view of Smith *et al.* (U.S. Publication No. 20020169162).

E. Whether Claim 55 is properly rejected under 35 U.S.C. § 103(a) as being obvious over Lehman *et al.*, *The Journal of Pediatrics, 140*:125-127 (2002) in view of Cardone *et al.*, *American Family Physician, 67*:2147-2152 (2003).

F. Whether Claim 1 is properly rejected under 35 U.S.C. § 103(a) as being obvious over Dunn (EP 1 153 606) in view of Braun and Sieper, *Expert Opin. Biol. Ther.* 3(1): 141-168 (2003).

G. Whether Claims 38 and 48 are properly rejected under 35 U.S.C. § 112, second paragraph as being indefinite.

H. Whether Claims 38 and 48 are properly rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the enablement requirement.

I. Whether Claim 46 is properly rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the enablement requirement.

J. Whether Claim 49 is properly rejected under 35 U.S.C. § 112, first paragraph as failing to comply with the enablement requirement.

K. Whether Claim 49 is properly rejected under 35 U.S.C. § 112, first paragraph as failing to comply with the written description requirement.

VII. ARGUMENT

A. CLAIMS 1, 2, 34, 37, 47, 49, 51, 54 AND 56 ARE NOT PROPERLY REJECTED UNDER 35 U.S.C. §103(a) AS BEING OBVIOUS OVER LEHMAN *ET AL., THE JOURNAL OF PEDIATRICS, 140*:125-127 (2002) IN VIEW OF DUNN (EP 1 153 606).

Appellants' independent claim, Claim 1, is directed to a method of treating an inflamed orthopedic joint comprising trans-capsularly administering an inhibitor of TNF-α synthesis. Claims 2, 34, 37, 47, 49, 51, 54 and 56 depend upon Claim 1, and, therefore, contain the same limitation.

The Examiner states that "[i]t would have been obvious to the person of ordinary skill in the art at the time the invention was made to modify the formulation comprising thalidomide taught by Lehman et al. using the administration route taught by Dunn". (see page 7 of Office Action mailed January 4, 2006 (First Office Action) and page 13 of Office Action mailed June 27, 2006 (Second Office Action)).

To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in some knowledge generally available in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of

success must be found in the prior art and not based in applicant's disclosure. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991). *Manual of Patent Examining Procedure* § 706.02(j).

Appellants' invention is directed to a method of treating an inflamed orthopedic joint comprising trans-capsularly administering into the joint space an inhibitor of TNF-α synthesis. As taught in Appellants' specification, direct administration of the inhibitor of TNF-α synthesis trans-capsularly is advantageous over systemic treatment. Such advantages include, for example, arresting the inflammation process begun within the joint and the degeneration of the hyaline cartilage, preventing intracapsular nerve irritation, increasing the half life of the inhibitor of TNF-α synthesis in the capsule and reducing unwanted side effects (see the specification, for example at page 8, line 10 to page 10, line 5).

Lehman *et al.* teaches that two children with systemic onset juvenile rheumatoid arthritis were systemically treated with etanercept (ENBREL®) and thalidomide. The treatment with etanercept was unsuccessful. The treatment with thalidomide demonstrated improvements in arthritis manifestations and laboratory parameters. However, Lehman *et al.* teaches that thalidomide has been shown to have both stimulatory and inhibitory effects on TNF-α activity, and notes that it may increase TNF-α production under some circumstances (see Lehman *et al.* at page 126, column 3). Further, Lehman *et al.* cites reference number fourteen (14), Gori *et al.*, "Tumor Necrosis Factor-α Increased Production During Thalidomide Treatment in Patients with Tuberculosis and Human Immunodeficiency Virus Coinfection", *J. Infect. Dis. 182*:639-640 (2000). Gori *et al.* states that reported data suggest that thalidomide is *not* a systemic TNF-α inhibitor. In fact, Gori *et al.* reported a progressive increase in TNF-α production following thalidomide treatment, concluding "we confirm that thalidomide did not reduce TNF-α levels...." (page 639). Further, Gori *et al.* states that, in light of these results, they suggest "extreme caution" in undertaking studies that support clinical use of thalidomide. *Id.* The Examiner states that the relevance of Gori is unclear because Gori is directed to treating a different population of patients. However, Lehman *et al.*'s citation of Gori indicates that Lehman *et al.* believes Gori's research is relevant to Lehman *et al.*'s findings.

The Examiner states that Appellants' specification teaches that thalidomide is among the compounds which prevent and/or inhibit TNF synthesis. However, what the specification actually discloses is that TNF antagonists include "compounds which prevent and/or inhibit TNF

synthesis, TNF release or its action on target cells, such as thalidomide, tenidap, phosphodiesterase inhibitors (e.g, pentoxifylline and rolipram), A2b adenosine receptor agonists and A2b adenosine receptor enhancers...." (page 15, lines 21-24). One of skill in the art would not conclude that thalidomide is an inhibitor of TNF-α synthesis, as recited in the claims.

Therefore, Lehman *et al.* does not teach or suggest administration via trans-capsular injection, and does not teach or suggest treating an inflamed orthopedic joint with an inhibitor of TNF-α synthesis. In fact, Lehman *et al.* teaches away from administration of an inhibitor of TNF-α synthesis in favor of a substance known to have at least some stimulatory effects on TNF-α activity (thalidomide). A prior art reference must be considered in its entirety, including portions that would lead away from the claimed invention. *W.L. Gore & Associates, Inc. v. Garlock, Inc.*, 721 F.2d 1540, 220 USPQ 303 (Fed. Cir. 1983), *cert. denied*, 469 U.S. 851 (1984), MPEP § 2142.02 (VI). Because thalidomide can increase TNF-α synthesis, Lehman *et al.* does not inherently teach treatment with an inhibitor of TNF-α synthesis. Moreover, even if it did, it does not teach or suggest local or trans-capsular administration. Thus, Lehman *et al.* does not describe or suggest Appellants' invention, and does not provide a reasonable expectation of successfully treating an inflamed orthopedic joint by trans-capsularly administering into the joint space an inhibitor of TNF-α synthesis.

Dunn teaches treating an inflamed joint by injecting growth hormone and buffer solution into the joint space. (See Dunn, column 3, paragraph 0009 and 0011). According to Dunn, the hormone is injected into the joint space and not directly into the bone, and in this manner it may flow over the entire joint surface and react with the vascular units at the bone-cartilage interface, and it may be absorbed into the bloodstream resulting in systemic effects, such as stimulation of production of bone marrow outside the joint. (See Dunn, column 7, paragraph 0027). The purpose of injection of the growth factor into the joint is to stimulate the articular growth plate at the joint surface. (See Dunn column 5, paragraph 0021 to column 6, paragraph 0023).

Dunn further discloses that, as a "preliminary" step, agents such as anti-kinases, growth factors and anti-cytokines including ENBREL® can be injected or otherwise applied to the joint prior to, or simultaneously with, the step of injecting a growth hormone and buffer solution into the joint space. (See Dunn abstract; column 8, paragraph 0030; and column 9, paragraph 0031). According to Dunn, the presence of these agents has the effect of "quieting" the joint due to the reduction or removal of the irritating activity of certain agents, e.g., TNF, which might impede or

impair the responsiveness of the joint to subsequent treatment with growth hormone for inflammation. ENBREL® does not inhibit TNF-α synthesis. Thus, Dunn does not describe or suggest Appellants' methods of administration of an inhibitor of TNF-α synthesis.

Even if thalidomide were inherently an inhibitor of TNF-α synthesis, one of skill in the art would not be motivated to substitute an inhibitor of TNF-α synthesis for the growth factor in Dunn to *trans-capsularly* administer an inhibitor of TNF-α synthesis on the basis of Lehman *et al.*'s teachings of *systemic* administration of thalidomide.

Further at the time of Appellants' invention, one of skill in the art would not have been motivated to locally administer any inhibitor of TNF-α synthesis. The state of the art was not to administer such compounds trans-capsularly. For example, as noted in Appellants' specification, at least one published application teaches in its examples that TNF inhibitors are to be administered through systemic pathways. Appellants' specification discloses that, in particular, a cited published application teaches that "the major contribution of TNF-alpha may be derived from recruited, aggregated and maybe even extravasated leukocytes, and that successful pharmacologic block may be achieved only by systemic treatment" (Appellants' specification, page 4, lines 8-17).

Thus, one of ordinary skill in the art would not be motivated to combine the teachings of Lehman *et al.* and Dunn with any reasonable expectation of success in treating an inflamed orthopedic joint by trans-capsularly administering an inhibitor of TNF-α synthesis, as claimed by Appellants. None of the Examiner's cited references alone or in combination teach or suggest the claimed invention, and the claimed invention is not obvious.

B. CLAIMS 36, 39-43, 45, 58, 60, 61, 63-65 ARE NOT PROPERLY REJECTED UNDER 35 U.S.C. § 103(a) AS BEING OBVIOUS OVER LEHMAN *ET AL., THE JOURNAL OF PEDIATRICS, 140*:125-127 (2002) IN VIEW OF PIKE *ET AL.* (US PUBLICATION NO. 20030134792).

Claims 36, 39-43, 45, 58, 60, 61 and 63-65 recite various aspects of administration. They depend upon Claim 1, and, therefore, are also directed to a method of treating an inflamed orthopedic joint comprising trans-capsularly administering an inhibitor of TNF-α synthesis. The Examiner states that these claims are obvious over Lehman *et al.* in view of Pike *et al.* However,

none of the Examiner's cited references alone or in combination teach or suggest treating an inflamed orthopedic joint comprising trans-capsularly administering an inhibitor of TNF-α synthesis.

As discussed above, Lehman *et al.* does not teach or suggest administration via trans-capsular injection of an inflamed orthopedic joint with an inhibitor of TNF-α synthesis, nor does it teach the different aspects of administration disclosed in the rejected claims. Pike *et al.* discloses the treatment of articular cartilage disorders by administering IGF-1, a growth factor, to preserve existing cartilage tissues or stimulate regeneration of cartilage. Such treatment includes administering IGF-1 by, for example, intra-articular injection. Although Pike *et al.* teaches that additional agents such as antibodies and anti-inflammatory agents can be included in its composition, Pike *et al.* does not teach or suggest administering an inhibitor of TNF-α synthesis, or any anti-cytokine agent. Thus, neither Lehman *et al.* nor Pike *et al.* describe or suggest Appellants' invention, and do not provide a reasonable expectation of treating an inflamed orthopedic joint by trans-capsularly administering an inhibitor of TNF-α synthesis.

The teaching or suggestion to make the claimed combination and the reasonable expectation of success must be found in the prior art and not based on Appellant's disclosure. Thus, for these reasons and the reasons discussed above, one of skill in the art would not be motivated to substitute an inhibitor of TNF-α synthesis for the growth factor in Pike *et al.* on the basis of Lehman *et al.*'s teachings of systemic administration of thalidomide. One of ordinary skill in the art would not be motivated to combine the teachings of Lehman *et al.* and Pike *et al.* with any reasonable expectation of success in treating an inflamed orthopedic joint with an inhibitor of TNF-α synthesis, as claimed by Appellants. None of the Examiner's cited references alone or in combination teach or suggest the claimed invention, and the claimed invention is not obvious.

C. CLAIM 50 IS NOT PROPERLY REJECTED UNDER 35 U.S.C. § 103(a) AS BEING OBVIOUS OVER LEHMAN *ET AL., THE JOURNAL OF PEDIATRICS, 140*:125-127 (2002) IN VIEW OF PIKE *ET AL.* (US PUBLICATION NO. 20030134792), AND MOLLOY *ET AL., SPORTS MED., 33*:381-394 (2003).

As noted above, Appellants' invention is directed to a method of treating an inflamed orthopedic joint comprising trans-capsularly administering a formulation comprising an inhibitor of TNF-α synthesis. Claim 50 is directed to the method wherein the formulation further comprises a growth factor provided by platelet concentrate. The Examiner states that this claim is obvious over Lehman *et al.* in view of Pike *et al.* and Molloy *et al* (first and second Office Actions). However, none of the Examiner's cited references alone or in combination teach or suggest treating an inflamed orthopedic joint comprising trans-capsularly administering an inhibitor of TNF-α synthesis.

For the reasons discussed above, neither Lehman *et al.* nor Pike *et al.*, alone or in combination teach or suggest treating an inflamed orthopedic joint comprising trans-capsularly administering an inhibitor of TNF-α synthesis. Nor does Lehman *et al.* teach a formulation further comprises a growth factor provided by platelet concentrate. Molloy *et al.* teaches that growth factors play a role in tendon healing. Molloy *et al.* does not teach or suggest Appellants' claimed methods of trans-capsular administration. Molloy *et al.* does not teach or suggest administering an inhibitor of TNF-α synthesis or any cytokine inhibitor. Thus, Molloy *et al.* does not describe or suggest Appellants' invention.

One of skill in the art would not be motivated to substitute the thalidomide of Lehman *et al.* into the method of Pike *et al.* to arrive at the invention claimed in Claim 50. Nothing in Mollowy's teachings about growth factors supplies the necessary motivation to combine the references to arrive at the claimed invention. Thus, one of ordinary skill in the art would not be motivated to combine the teachings of Lehman *et al.*, Pike *et al.* and Molloy *et al.* with any reasonable expectation of success in treating an inflamed orthopedic joint by trans-capsularly administering into the joint space an inhibitor of TNF-α synthesis. The claimed invention is not obvious.

D. CLAIMS 1, 53 AND 57 UNDER 35 U.S.C. § 103(a) ARE NOT PROPERLY REJECTED UNDER 35 U.S.C. § 103(a) AS BEING OBVIOUS OVER LEHMAN *ET AL., THE JOURNAL OF PEDIATRICS, 140*:125-127 (2002) IN VIEW OF SMITH *ET AL.* (U.S. PUBLICATION NO. 20020169162).

As noted above, Appellants' invention is directed to a method of treating an inflamed orthopedic joint comprising trans-capsularly administering a formulation comprising an inhibitor

of TNF-α synthesis. Claim 53 is directed to the method of Claim 1, wherein the formulation is injected into the synovial fluid. Claim 57 is directed to the method of Claim 1, wherein the formulation is administered through a drug pump.

The Examiner states that these claims are obvious over Lehman *et al.* in view of Smith *et al.* (first and second Office Actions). However, none of the Examiner's cited references alone or in combination teach or suggest treating an inflamed orthopedic joint comprising trans-capsularly administering an inhibitor of TNF-α synthesis. As discussed above, Lehman *et al.* teaches systemic administration of thalidomide. Lehman *et al.* does not teach or suggest trans-capsularly administering an inhibitor of TNF-α synthesis in the synovial fluid-containing portion of the joint, or administering an inhibitor of TNF-α synthesis through a drug pump.

Smith *et al.* teaches a sustained release device which may be surgically implanted intraarticularly, *i.e.*, within the synovial joint. (See Smith *et al.* at paragraph 0046). According to Smith *et al.*, the sustained release device is capable of releasing drugs or compounds over an extended period of time in a controlled fashion, as opposed to repeated injections (See Smith *et al.* at paragraphs 0012 and 0047). Smith *et al.* teaches that the compounds that can be administered via the sustained release device include glucocorticoids, anti-inflammatories such as dexamethasone, fluocinolone, cortisone, prednisolone, flumetholone, and derivatives thereof; non-steroidal anti-inflammatory drugs and cyclosporines. (See Smith at paragraph 0043). Smith *et al.* does not teach or suggest administering an inhibitor of TNF-α synthesis. Thus, neither Lehman *et al.* nor Smith *et al.* describes or suggests Appellants' invention. One of skill in the art would not be motivated by Lehman *et al.*'s systemic administration of thalidomide to substitute an inhibitor of TNF-α synthesis into Smith *et al.*'s methods.

One of ordinary skill in the art would not be motivated to combine the teachings of Lehman *et al.* and Smith *et al.* with any reasonable expectation of success in treating an inflamed orthopedic joint, by trans-capsular administration of an inhibitor of TNF-α synthesis either by synovial fluid injection or by drug pump as claimed by Appellants. Thus, none of the references cited by the Examiner alone or in combination teach or suggest the claimed invention, and the claimed invention is not obvious.

E. CLAIM 55 IS NOT PROPERLY REJECTED UNDER 35 U.S.C. § 103(a) AS BEING OBVIOUS OVER LEHMAN *ET AL., THE JOURNAL OF PEDIATRICS,*

140:125-127 (2002) IN VIEW OF CARDONE *ET AL., AMERICAN FAMILY PHYSICIAN, 67*:2147-2152 (2003).

Claim 55 is directed to a method of treating an inflamed orthopedic joint comprising trans-capsularly administering a formulation comprising inhibitor of TNF-α synthesis wherein a portion of the synovial fluid is removed prior to administration of the inhibitor of TNF-α synthesis. The Examiner states that this claim is obvious over Lehman *et al.* in view of Cardone *et al.* However, none of the references cited by the Examiner alone or in combination teach or suggest the claimed invention (first Office Action).

As discussed above, Lehman *et al.* does not teach or suggest Appellants' invention. Cardone *et al.* teaches injection procedures for administering corticosteroids into the hip and knee joints as diagnostic and therapeutic tools. In addition, Cardone *et al.* teaches aspiration procedures for the knee for the purpose of diagnosing an unexplained effusion and to relieve discomfort caused by the effusion. Cardone *et al.* does not teach or suggest removing a portion of the synovial fluid prior to trans-capsular administration of an inhibitor of TNF-α synthesis. Thus, Cardone *et al.* does not describe or suggest Appellants' invention.

One of ordinary skill in the art would not be motivated to combine the teachings of Lehman *et al.* and Cardone *et al.* with any reasonable expectation of success in treating an inflamed orthopedic joint by trans-capsularly administering into the joint space an inhibitor of TNF-α synthesis. Thus, none of the references cited by the Examiner alone or in combination teach or suggest the claimed invention, and the claimed invention is not obvious.

F. CLAIM 1 IS NOT PROPERLY REJECTED UNDER 35 U.S.C. § 103(a) AS BEING OBVIOUS OVER DUNN (EP 1 153 606) IN VIEW OF BRAUN AND SIEPER,, *EXPERT OPIN. BIOL. THER.* 3(1): 141-168 (2003).

As noted above, Appellants' invention is directed to a method of treating an inflamed orthopedic joint comprising trans-capsularly administering a formulation comprising an inhibitor of TNF-α synthesis.

As discussed in detail above, Dunn does not describe trans-capsular administration of an inhibitor of TNF-α synthesis, and does not teach the invention of Claim 1. Braun teaches use of infliximab, a chimeric anti-TNF antibody, to treat rheumatoid arthritis by single *intravenous* (i.e.,

systemic) infusions. It does not teach or suggest trans-capsular administration into a joint space, nor does it teach any potential value of any local administration. One of skill in the art would not have been motivated to substitute infliximab in the methods of Braun with a reasonable expectation of success. The motivation to combine must come from the references themselves and not from the benefit of hindsight based on teachings in the Appellants' specification. Thus, the claimed invention is not obvious.

G. CLAIMS 38 AND 48 ARE NOT PROPERLY REJECTED UNDER 35 U.S.C. §112, SECOND PARAGRAPH AS BEING INDEFINITE.

Claim 38 is directed to the method of Claim 1, wherein the inhibitor of TNF-α synthesis is present in the formulation in an amount of at least 100 mg/ml. Claim 48 is directed to the method of Claim 1, wherein the inhibitor of TNF-α synthesis is present in the formulation in a maximum amount of 0.5 mg. The Examiner has rejected these claims as indefinite, stating that "[i]n the absence of a specific recited structure, the recitation of a specific dosage is meaningless." (first Office Action).

With regard to the definiteness requirement of 35 U.S.C. § 112, second paragraph, the Examiner's focus during examination of the claims for compliance with the requirement for definiteness is "whether the claim meets the threshold requirements of clarity and precision...." See *Manual of Patent Examining Procedure* (MPEP) §2173.02.

> When the examiner is satisfied that patentable subject matter is disclosed, and it is apparent to the examiner that the claims are directed to such patentable subject matter, he or she should allow claims which define the patentable subject matter with a reasonable degree of particularity and distinctness. Some latitude in the manner of expression and the manner of terms should be permitted even though the claim language is not as precise as the examiner might desire.

Id. (emphasis in original)

Claims 38 and 48 recite administration of particular amounts of "an inhibitor of TNF-α synthesis." Since none of the other claims reciting "an inhibitor of TNF-α synthesis" are rejected as indefinite, it appears that the rejection is directed to the use of amounts. Definiteness of claim language must be analyzed not in a vacuum, but in light of the claim interpretation that

would be given by one possessing the ordinary level of skill in the pertinent art at the time the invention was made. *Id.* One of ordinary skill in the art should understand what is meant by amounts of such inhibitors in units such as "mg/ml" and of "mg". Thus, it would be very straightforward for one of ordinary skill in the art to understand what is meant by an inhibitor of TNF-α synthesis "present in the formulation in an amount of at least 100 mg/ml" and "present in the formulation in a maximum amount of 0.5 mg" (see, for example, the specification at page 22, line 10 to page 23, line 21). Thus, each claim "apprises one of ordinary skill in the art of its scope and, therefore, serves its notice function...." and "defines the patentable subject matter with reasonable degree of particularity and distinctness." *Id.* The claims are definite.

H. CLAIMS 38 AND 48 ARE NOT PROPERLY REJECTED UNDER 35 U.S.C. § 112, FIRST PARAGRAPH, AS FAILING TO COMPLY WITH THE ENABLEMENT REQUIREMENT.

The Examiner has also rejected Claims 38 and 48 as not enabled, stating "[i]n the absence of a specific recited structure the skilled artisan is unable to make the recited compound" (first Office Action). As indicated above, Claims 38 and 48 recite administration of particular amounts of an inhibitor of TNF-α synthesis. The claims do not require the artisan to make the compounds; they merely require the artisan to measure them. Otherwise, these claims do not differ from Claim 1, which is not rejected for enablement. The level of skill is the art is high. Inhibitors of TNF-α synthesis are well-known and available in the art, as are procedures to measure them. One of skill in the art could easily determine how to measure an inhibitor of TNF-α synthesis in a formulation in an amount of at least 100 mg/ml or in a formulation in a maximum amount of 0.5 mg without undue experimentation. (see, for example, the specification at page 22, line 10 to page 23, line 21). The claims are enabled.

I. CLAIM 46 IS NOT PROPERLY REJECTED UNDER 35 U.S.C. § 112, FIRST PARAGRAPH, AS FAILING TO COMPLY WITH THE ENABLEMENT REQUIREMENT.

Claim 46 recites "wherein the sustained release device comprises an inflammatory-responsive delivery system." The Examiner rejects this claim, stating that "[t]he specification provides no guidance and/or direction or working examples of a sustained release device which could deliver a formulation comprising an effective amount of an inhibitor of TNF-α synthesis." In addition, the Examiner states that LaVan *et al.* discloses that there are stability problems with *in vivo* glucose sensors.

Although LaVan *et al.* discloses that there are stability problems with *in vivo* glucose sensors, LaVan *et al.* does not disclose any such problems with a sustained release device that comprises an inflammatory-responsive delivery system. In fact, Pike *et al.* (US Publication No. 20030134792), which was cited by the Examiner in §103(a) rejections, states at paragraph 0053 that a sustained release device comprising inflammatory-responsive delivery systems is "well known in the art," and can be used to administer a therapeutically effective dose of an agent directly at the site. The Examiner states that LaVan *et al.* indicates that "smart" delivery devices require more safety and efficacy testing before clinical use, and "one could logically conclude that these systems were not yet reduced to practice." (second Office Action). However, enablement does not require reduction to practice or establishment of a particular degree of safety or efficacy. The proper standard is whether one of skill in the art can make or use the claimed invention without undue experimentation. The teachings in the specification, combined with what was known in the art, establish that this standard is met. Thus, Claim 46 is enabled.

J. CLAIM 49 IS NOT PROPERLY REJECTED UNDER 35 U.S.C. § 112, FIRST PARAGRAPH, AS FAILING TO COMPLY WITH THE ENABLEMENT REQUIREMENT.

Claim 49 recites "wherein the formulation further comprises a growth factor present in an amount effective to repair joint tissue." The Examiner states that Claim 49 is not enabled because the specification fails to teach the skilled artisan how to use the factors recited without undue experimentation to determine whether a given protein would be useful in the claimed methods and what the dosage would be (second Office Action). The specification discusses the factors in detail (e.g., page 32, line 11-page 33, line 2). The level of skill in the relevant art is high, and therapeutic administration of growth factors was well-known at the time of the

invention (see, for example, Dunn *et al.* and Pike *et al.* above). A skilled practitioner could easily determine whether an inflamed knee joint is being treated, and how to determine appropriate dosage of known factors for such treatment, with only routine experimentation, the claim is enabled.

K. CLAIM 49 IS NOT PROPERLY REJECTED UNDER 35 U.S.C. § 112, FIRST PARAGRAPH, AS FAILING TO COMPLY WITH THE WRITTEN DESCRIPTION REQUIREMENT.

Claim 49 recites "wherein the formulation further comprises a growth factor present in an amount effective to repair joint tissue." The Examiner has rejected this claim as failing to meet these written description requirement on the grounds that the specification does not "clearly allow persons of ordinary skill in the art to recognize [he or she] invented what is now claimed [with regard to "growth factors]" (second Office Action).

As noted above, at the time of the invention, therapeutic administration of growth factors may was well known. As noted by the Examiner, the specification lists a myriad of growth factors that may be used in the invention (see, e.g., page 32, line 11-page 33, line 2). As further noted by the Examiner, the skilled artisan would be aware of a number of different compounds which would be classified under the heading "growth factors". Appellants are not required to put into a specification what is well known. Clearly, the claimed subject matter is described in the specification in a manner which does demonstrate that the Appellants had possession of the specific subject matter claimed, and the requirement for written description has been met.

In view of the foregoing arguments and legal authority, reversal of the rejections is requested.

Respectfully submitted,

HAMILTON, BROOK, SMITH & REYNOLDS, P.C.

By Christine M. Wise

Christine M. Wise
Registration No.: 58,073
Telephone: (978) 341-0036
Facsimile: (978) 341-0136

Concord, MA 01742-9133

Dated: May 30, 2007

CLAIMS APPENDIX

1. A method of treating an inflamed orthopedic joint, said joint comprising i) opposing hyaline cartilage articular surfaces, ii) a peripheral collagenous capsule defining a central joint space and iii) synovial fluid contained within the joint space, comprising trans-capsularly administering into the joint space a formulation comprising an effective amount of an inhibitor of TNF-α synthesis such that the inflamed orthopedic joint is treated.

2. The method of claim 1, wherein the joint is a knee joint.

34. The method of claim 1, wherein the formulation further comprises at least one growth factor.

36. The method of claim 1, wherein the formulation further comprises a liposomal delivery system.

37. The method of claim 1, wherein the formulation is administered in an amount of less than 1 cc.

38. The method of claim 1, wherein the inhibitor of TNF-α synthesis is present in the formulation in an amount of at least 100 mg/ml.

39. The method of claim 1, wherein the formulation further comprises a sustained release device.

40. The method of claim 39, wherein the sustained release device comprises a hydrogel.

41. The method of claim 39, wherein the sustained release device provides controlled release.

42. The method of claim 39, wherein the sustained release device provides continuous release.

43. The method of claim 39, wherein the sustained release device provides intermittent release.

45. The method of claim 39, wherein the sustained release device comprises microspheres having a plurality of degradation rates.

46. The method of claim 39, wherein the sustained release device comprises an inflammatory-responsive delivery system.

47. The method of claim 1, wherein the formulation is provided closely adjacent to the outer wall of the capsule.

48. The method of claim 1, wherein the inhibitor of TNF-α synthesis is present in the formulation in a maximum amount of 0.5 mg.

49. The method of claim 1, wherein the formulation further comprises a growth factor present in an amount effective to repair joint tissue.

50. The method of claim 49, wherein the growth factor is provided by platelet concentrate.

51. The method of claim 1, wherein the inhibitor of TNF-α synthesis therapeutically inhibits the production of a cytokine.

53. The method of claim 1, wherein the formulation is injected into the synovial fluid.

54. The method of claim 1, wherein the formulation includes a viscosupplement.

55. The method of claim 1, wherein a portion of the synovial fluid is removed prior to administration of the inhibitor of TNF-α synthesis.

56. The method of claim 1, wherein the administration is performed through a needle.

57. The method of claim 1, wherein the formulation is administered through a drug pump.

58. The method of claim 1, wherein the formulation is administered in a volume of between 0.03 ml and 0.3 ml.

60. The method of claim 1, wherein the administration comprises providing the formulation in a patch attached to an outer wall of the capsule.

61. The method of claim 1, wherein the administration comprises providing the formulation in a depot at a location closely adjacent an outer wall of the capsule.

63. The method of claim 1, wherein the inhibitor of TNF-α synthesis is predominantly released from the formulation by diffusion of the high specificity antagonist through a sustained delivery device.

64. The method of claim 63, wherein the sustained delivery device is a polymer.

65. The method of claim 1, wherein the inhibitor of TNF-α synthesis is predominantly released from the formulation by biodegradation of a sustained delivery device.

EVIDENCE APPENDIX

1. Lehman *et al.*, "Thalidomide Therapy for Recalcitrant Systemic Onset Juvenile Rheumatoid Arthritis," *J. Pediatrics, 140*:125-127 (2002).

   Lehman *et al.* was listed by the Examiner as Reference V on form PTO-892 accompanying the Office Action dated January 4, 2006.

2. Dunn, EP 1 153607 A2, Publication Date: November 14, 2001.

   Dunn was listed by the Examiner as Reference N on form PTO-892 accompanying the Office Action dated January 4, 2006.

3. Pike *et al.*, US 2003/0134792, Publication Date: July 17, 2003.

   Pike *et al.* was listed by the Examiner as Reference A on form PTO-892 accompanying the Office Action dated January 4, 2006.

4. Molloy *et al.*, "*The* Roles of Growth Factors in Tendon and Ligament Healing," *Sports Med.*, 33:381-394 (2003).

   Molloy *et al.* was listed by the Examiner as Reference X on form PTO-892 accompanying the Office Action dated January 4, 2006.

5. Gori *et al.*, "Tumor Necrosis Factor-α Increased production During Thalidomide Treatment in Patients with Tuberculosis and Human Immunodeficiency Virus Coinfection," *J. Infect. Dis. 182*:639-640 (2000).

   Gori *et al.* was provided as Exhibit A with the Amendment entered into the record on April 4, 2006.

6. Smith *et al.*, US 2002/0169162, Publication Date: November 14, 2002.

   Smith *et al.* was listed by the Examiner as Reference B on form PTO-892 accompanying the Office Action dated January 4, 2006.

7. Cardone *et al.*,"Diagnostic and Therapeutic Injection of the Hip and Knee," *American Family Physician,* 67: 2147-2152 (2003).

   Cardone *et al.* was listed by the Examiner as Reference U on form PTO-892 accompanying the Office Action dated January 4, 2006.

8. LaVan, *et al.*, "Small-scale Systems for In Vivo Drug Delivery," *Nature Biotechnology 21*: 1184-1191 (2003).

   LaVan was listed by the Examiner Reference U on form PTO-892 accompanying the Office Action dated January 4, 2006.

9. Braun, J. and Sieper, J., "Overview of The Use of The Anti-TNF Agent Infliximab in Chronic Inflammatory Diseases," *Expert Opin. Biol. Ther. 3*(1):141-168 (2003).

   Braun, J. and Sieper, J. was listed by the Examiner Reference V on form PTO-892 accompanying the Office Action dated June 27, 2006.

RELATED PROCEEDINGS

NONE

Thalidomide therapy for recalcitrant systemic onset juvenile rheumatoid arthritis

Thomas J. A. Lehman, MD, Kim H. Striegel, FNP, and Karen B. Onel, MD

Systemic onset juvenile rheumatoid arthritis unresponsive to nonsteroidal anti-inflammatory drugs may be controlled with corticosteroids, but these drugs have significant side effects. We report 2 steroid-dependent children with systemic onset juvenile rheumatoid arthritis who did not respond to multiple nonsteroidal anti-inflammatory drugs, methotrexate, azathioprine, cyclosporine, and etanercept. Both children had significant improvement with thalidomide therapy. (J Pediatr 2002;140:125-7)

Systemic onset juvenile rheumatoid arthritis (SoJRA) is often a severe debilitating condition accompanied by fever, rash, leukocytosis, anemia, and elevated erythrocyte sedimentation rate.[1] Although some children respond well to nonsteroidal anti-inflammatory drugs and others may be controlled with second-line agents, approximately one third of children are dependent on corticosteroids for control of the systemic manifestations of the disease. Severe cases of SoJRA may be life threatening.[1] Some children with SoJRA have severe macrophage activation syndrome, whereas others have a lifetime of chronic debilitation because of the combined effects of the disease and chronic corticosteroid dependence. The side effects of corticosteroids in growing children often result in permanent physical and psychologic disabilities.[2]

To reduce their dependence on corticosteroids, children with severe SoJRA are often treated with intravenous γ globulin, methotrexate, cyclosporine, azathioprine, etanercept, and/or cyclophosphamide or autologous stem cell transplantation in varying combinations and without consistent results.[3-7] There is a great need for an effective agent with less risk of morbidity and mortality for children with severe SoJRA.

ESR	Erythrocyte sedimentation rate
Hgb	Hemoglobin
IFN	Interferon
TNF	Tumor necrosis factor
SoJRA	Systemic onset juvenile rheumatoid arthritis

Thalidomide is a unique therapy for inflammation that appears to reverse the specific pattern of cytokine abnormalities that has been noted in children with SoJRA.[8] We report 2 children with severe SoJRA who failed treatment with nonsteroidal anti-inflammatory drugs, methotrexate, cyclosporine, and etanercept, but who who had improvement with thalidomide therapy. This allowed us to gradually reduce their corticosteroids to an acceptable level and withdraw the majority of their other immunosuppressive medications.

Case Histories

A 7.5-year-old white boy had a 3-year history of SoJRA. He had (1) fever spikes to 39.5°C, with the temperature returning to normal at least once each day, (2) arthritis in the wrists, knees, and neck, and (3) a fleeting salmon pink rash. The initial erythrocyte sedimentation rate (ESR) was 120 mm/hour and hemoglobin (Hgb) was 9.6 g/dL, with a white blood cell count of 30,000 cells/mm^3. He was treated with naproxen, then indomethacin and methotrexate (10 mg/M^2 per week) but required 9 mg prednisone daily (0.3 mg/kg) to allow activities of daily living. The disease continued to progress; the prednisone was increased to 30 mg daily (1 mg/kg) and etanercept was added. In the absence of clinical response, the etanercept was increased to 25 mg by subcutaneous injection twice weekly (0.85 mg/kg). After 1 year the etanercept was discontinued, the methotrexate was increased to 15 mg weekly, and the prednisone was increased to 40 mg/day. The active disease continued with fever, rash, and polyarthritis. Hgb was 9.3 g/dL, ESR was 123 mm/h, and

From the Division of Pediatric Rheumatology, Hospital for Special Surgery, and the Department of Pediatrics, Sanford Weill Medical Center of Cornell University, New York.

Submitted for publication Mar 30, 2001; revisions received June 11, 2001, and Oct 2, 2001; accepted Oct 6, 2001.

Reprint requests: Thomas J. A. Lehman, MD, Division of Pediatric Rheumatology, Hospital for Special Surgery, Sanford Weill Medical Center, Cornell University, 535 E 70th St, New York, NY 10021.

0022-3476/2002/$35.00 + 0 9/22/120835

doi:10.1067/mpd.2002.120835

white blood cell count was 22,300 cells/mm^3. Cyclosporine (100 mg twice a day, 5 mg/kg) and 75 mg azathioprine (1.75 mg/kg/day) were added during the next 6 months with no clinical response. In June 2000, the Hgb was 9.6 g/dL, ESR was 60 mm/hour, and white blood cell count was 14,000 cells/mm^3. Fever, rash, and polyarthritis involving both wrists, both shoulders, both knees, and the right hip continued. After we obtained informed consent, we started treatment with thalidomide at 2.5 mg/kg/day (100 mg daily). By October 2000, the Hgb was 11.5 g/dL, ESR was 10 mm/h, and white blood cell count was 12,000 cells/mm^3. The daily fever spikes resolved, the rash disappeared, and the arthritis resolved with the exception of persistent limitation in the range of motion of the right wrist and right hip. During the ensuing months, the prednisone was reduced to 7.5 mg per day, the azathioprine was withdrawn, and we planned to withdraw the cyclosporine. He continues to receive 15 mg per week of methotrexate, with Hgb of 11.3 g/dL, ESR of 10 mm/hour, and a white blood cell count of 9600 cells/mm^3.

A 15-year-old white boy with severe SoJRA at 11 years was examined. He had fever spikes that returned to normal each day, salmon pink rash, and arthritis. He had been treated with tolmetin sodium 30 mg/kg/day and prednisone 0.5 mg/kg/day. While he was taking prednisone, insulin-dependent diabetes mellitus developed. When first seen, he was taking prednisone 20 mg/day (0.67 mg/kg/day), hydroxychloroquine 200 mg/day, tolmetin sodium 40 mg/kg/day, and insulin. The Hgb was 11.5 g/dL, ESR was 51 mm/h, and white blood cell count was 18,600 cells/mm^3. Tolmetin sodium was discontinued. Indomethacin (3 mg/kg/day) and sulfasalazine (60 mg/kg/day) were added. The prednisone was reduced to 5 mg daily, and insulin injections were discontinued. After 6 months, the arthritis progressed with bilateral wrist and knee swelling and rash. Methotrexate was recommended but refused by the family. Etanercept was added without improvement. After 12 weeks, the etanercept was withdrawn and 3 mg/kg/day cyclosporine was added. In June 2000, the Hgb was 10.5 g/dL, ESR was 55 mm/h, and white blood cell count was 7600 cells/mm^3. In July, he had recurrent fever, rash, and widespread arthritis, which did not respond to an increase of the cyclosporine to 10 mg/kg/day. Prednisone was increased to 7.5 mg/day, and insulin injections were resumed. The patient then had avascular necrosis of the left hip. Despite the continuing active disease, the family refused to increase his prednisone dose because of difficulty controlling the diabetes. In September, he had persistent gross hematuria. Indomethacin and cyclosporine were withdrawn. After we obtained informed consent, thalidomide was added at 150 mg/day (3 mg/kg). After 4 months, the prednisone was reduced to 2.5 mg/day, and he continues to take sulfasalazine and hydroxychloroquine. He has no active arthritis, fever, or rash. The Hgb was 11.5 g/dL, ESR was 2 mm/h, and white blood cell count was 5500 cells/mm^3.

Both of these children had severe SoJRA that was resistant to multiple drugs and did not respond to a minimum of 8 weeks of etanercept. Both had dramatic improvements in arthritic manifestations and laboratory parameters equivalent to a greater than American College of Rheumatology 70 response within 3 months of initiating therapy with thalidomide. Both have been able to substantially reduce the prednisone dosage and withdraw all or most immunosuppressive medications.

DISCUSSION

Both of the children had failed multiple drug regimens before beginning treatment with thalidomide. Both had long-term corticosteroid therapy with long-term side effects and were considered potential candidates for autologous stem cell transplantation. However, both had significant improvement after beginning thalidomide therapy. Furthermore, both children have been able to reduce the corticosteroid dosage and withdraw other immunosuppressive medications. It is unlikely that thalidomide was effective only because of its effects on tumor necrosis factor (TNF)-α because therapy with etanercept was previously unsuccessful for both.

The etiopathogenesis of SoJRA is poorly understood. It shares only superficial similarities with the other diseases categorized as juvenile rheumatoid arthritis (juvenile idiopathic arthritis in the proposed new nomenclature) and has little if any relation to adult onset rheumatoid arthritis.[1] Previous studies have demonstrated a distinct cytokine profile in children with SoJRA.[8] Children with SoJRA manifest significant overproduction of TNF-α with decreased interferon (IFN)-γ production.[9] Some of the increased TNF-α production may be related to the association between specific TNF-α alleles related to high production and systemic onset juvenile rheumatoid arthritis.[10] During febrile episodes, high levels of the proinflammatory cytokine interleukin-6 are found in children with active SoJRA.[11] Increased levels of interleukin-8 and monocyte chemotactic protein-1[12] and adhesion molecules E-selectin and intracellular adhesion molecule-1 are also found in children with active SoJRA.[13]

Thalidomide appears to have multiple effects on the immune system and cytokine production that are not yet fully elucidated. It has been shown to have both stimulatory and inhibitory effects on TNF-α activity. It may increase TNF-α production under some circumstances, but it enhances the degradation of TNF-α mRNA, thereby reducing the half-life of TNF-α.[14] In chronically ill patients with human immunodeficiency virus and tuberculosis, thalidomide was shown to increase IFN-γ and interleukin-10 while decreasing TNF-α, interleukin-6, and interleukin-1β levels.[15] Thus, in this

clinical situation, it reverses the pattern of cytokine abnormalities seen in children with active SoJRA. In contrast to thalidomide, dexamethasone decreases IFN-γ and interleukin-10 production in a dose-dependent manner, thereby suppressing potentially beneficial as well as harmful cytokine effects.[16]

Thalidomide has been demonstrated to be effective in the treatment of a wide variety of diseases, including Crohn's disease and Behçet's syndrome.[17,18] It is interesting to note that thalidomide has not proven particularly helpful in the treatment of adult onset rheumatoid factor positive arthritis,[19] but this is a different condition. There is one case report describing the successful use of thalidomide for adult onset Still's disease.[20]

Although many physicians are familiar with the toxic effects of thalidomide use during pregnancy, the drug has not been shown to have any lingering effects on gestation occurring after it has been discontinued. A rigorous program of patient monitoring has been put into place by the manufacturer to ensure that every prescribing physician and patient are well aware of the possible side effects of the drug.

Large-scale controlled trials will be necessary to demonstrate the safety and efficacy of thalidomide in children with SoJRA.

REFERENCES

1. Lomater C, Gerloni V, Gattinara M, Mazzotti J, Cimaz R, Fantini F. Systemic onset juvenile idiopathic arthritis: a retrospective study of 80 consecutive patients followed for 10 years. J Rheumatol 2000;27:491-6.
2. Spahn JD, Kamada AK. Special considerations in the use of glucocorticoids in children. Pediatr Rev 1995;16:266-72.
3. Silverman ED, Cawkwell GD, Lovell DJ, Laxer RM, Lehman TJA, Passo MH, et al. Intravenous immunoglobulin in the treatment of systemic juvenile rheumatoid arthritis: a randomized placebo controlled trial. Pediatric Rheumatology Collaborative Study Group. J Rheumatol 1994;21:2353-8.
4. Wallace CA, Sherry DD. Trial of intravenous pulse cyclophosphamide and methylprednisolone in the treatment of severe systemic-onset juvenile rheumatoid arthritis. Arthritis Rheum 1997;40:1852-5.
5. Shaikov AV, Maximov AA, Speransky AI, Lovell DJ, Giannini EH, Solovyev SK. Repetitive use of pulse therapy with methylprednisolone and cyclophosphamide in addition to oral methotrexate in children with systemic juvenile rheumatoid arthritis: preliminary results of a long-term study. J Rheumatol 1992;19:612-6.
6. Gattorno M, Buoncompagni A, Faraci M, Pistoia V. Early treatment of systemic onset juvenile chronic arthritis with low-dose cyclosporin A. Clin Exp Rheumatol 1995;13:409-10.
7. Wulffraat N, van Royen A, Bierings M, Vossen J, Kuis W. Autologous haemopoietic stem-cell transplantation in four patients with refractory juvenile chronic arthritis. Lancet 1999;353:550-3.
8. Mangge H, Schauenstein K. Cytokines in juvenile rheumatoid arthritis (JRA). Cytokine 1998;10:471-80.
9. Muller K, Herner EB, Stagg A, Bendtzen K, Woo P. Inflammatory cytokines and cytokine antagonists in whole blood cultures of patients with systemic juvenile chronic arthritis. Br J Rheumatol 1998;37:562-9.
10. Date Y, Seki N, Kamizono S, Higuchi T, Hirata T, Miyata K, et al. Identification of a genetic risk factor for systemic juvenile rheumatoid arthritis in the 5'-flanking region of the TNF-α gene and HLA genes. Arthritis Rheum 1999;42:2577-82.
11. Fishman D, Faulds G, Jeffery R, Mohammed-Ali V, Yudkin JS, Humphries S, et al. The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. J Clin Invest 1998;102:1369-76.
12. De Benedetti F, Pignatti P, Bernasconi S, Gerloni V, Matsushima K, Caporali R, et al. Interleukin 8 and monocyte chemoattractant protein-1 in patients with juvenile rheumatoid arthritis: relation to onset types, disease activity, and synovial fluid leukocytes. J Rheumatol 1999;26:425-31.
13. De Benedetti F, Vivarelli M, Pignatti P, Olivieri M, Massa M, Pistorio A, et al. Circulating levels of soluble E-selectin, P-selectin and intercellular adhesion molecule-1 in patients with juvenile idiopathic arthritis. J Rheumatol 2000;27:2246-50.
14. Gori A, Rossi MC, Trabattoni D, Marchetti G, Fusi ML, Molteni C, et al. Tumor necrosis factor-α increased production during thalidomide treatment in patients with tuberculosis and human immunodeficiency virus coinfection. J Infect Dis 2000;182:639-40.
15. Corral LG, Haslett PA, Mulle GW, Chen R, Wong LM, Ocampo CJ, et al. Differential cytokine modulation and T cell activation by two distinct classes of thalidomide analogues that are potent inhibitors of TNF-α. J Immunol 1999;163:380-6.
16. Rowland TL, McHugh SM, Deighton J, Dearman RJ, Ewan PW, Kimber I. Differential regulation by thalidomide and dexamethasone of cytokine expression in human peripheral blood mononuclear cells. Immunopharmacology 1998;40:11-20.
17. Ehrenpreis ED, Kane SV, Cohen LB, Cohen RD, Hanauer SB. Thalidomide therapy for patients with refractory Crohn's disease: an open-label trial. Gastroenterology 1999;117:1271-7.
18. Gardner-Medwin JM, Smith NJ, Powell RJ. Clinical experience with thalidomide in the management of severe oral and genital ulceration in conditions such as Behcet's disease: use of neurophysiological studies to detect thalidomide neuropathy. Ann Rheum Dis 1994;53:828-32.
19. Keesal N, Wasserman MJ, Bookman A, Lapp V, Weber DA, Keystone EC. Thalidomide in the treatment of refractory rheumatoid arthritis. J Rheumatol 1999;26:2344-7.
20. Stambe C, Wicks IP. TNF-α and response of treatment-resistant adult-onset Still's disease to thalidomide. Lancet 1998;352:544-5.

(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 1 153 607 A2

(12) EUROPEAN PATENT APPLICATION

(43) Date of publication:
14.11.2001 Bulletin 2001/46

(51) Int Cl.[7]: **A61K 38/27**, A61P 19/02

(21) Application number: **01304200.7**

(22) Date of filing: **10.05.2001**

(84) Designated Contracting States:
AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE TR
Designated Extension States:
AL LT LV MK RO SI

(30) Priority: **10.05.2000 US 202561 P**
27.06.2000 US 214592 P
02.01.2001 US 778397

(71) Applicant: **Dunn, Allan Reuben**
North Miami, FL 33181 (US)

(72) Inventor: **Dunn, Allan Reuben**
North Miami, FL 33181 (US)

(74) Representative: **Maschio, Antonio et al**
D Young & Co,
21 New Fetter Lane
London EC4A 1DA (GB)

(54) Growth hormone for the treatment of joint inflammation

(57) Treating inflammation in a joint whether heat, redness, pain, swelling and/or stiffness, and for increasing motion and increasing joint space and correcting mal-alignment by injecting one time or multiple repeat times, a single dosage of a mixture of purified growth hormone (somatotropin) and buffer solution into the joint. The present invention also includes injecting or otherwise applying anti-cytokines, anti-kinases, and/or anti growth factors prior to, or simultaneously with, the step of injecting the mixture of purified growth hormone and buffer solution into the joint.

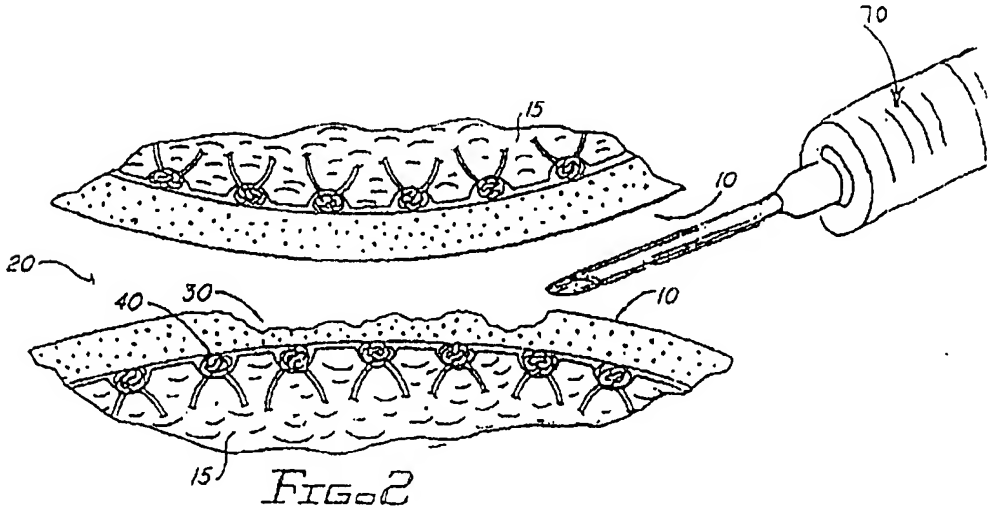

FIG. 2

EP 1 153 607 A2

Description

BACKGROUND OF THE INVENTION

[0001] The present invention relates to a process of treating inflammation in a joint, such as but not limited to a knee joint, a hip joint or an ankle joint, which has been damaged or which has otherwise become defective, and thereby, alleviating pain, heat, redness, swelling, stiffness, and other difficulties typically associated with a damaged or defective articular cartilage surface in a joint. More in particular, the present invention is directed to a process of preparing a joint prior to treatment for inflammation, as described herein, by injecting or otherwise applying a group of agents such as anti-cytokines, anti-kinases, anti growth factors - used individually or in various combinations thereof, to quiet and reduce deleterious activity in the joint prior to the step of injecting a mixture of purified growth hormone (commonly known as somatotropin) and a buffer solution into the joint of a body, preferably but not limited to that of a human, so as to initiate the treatment process.

[0002] The ends of bones which form a joint, including vertebra, are covered by articular cartilage, which is a thin, fragile tissue layer and which allows the bone ends to move freely and without pain. Many arthritic diseases and many degrees of trauma can, however, cause destruction or deterioration of this fragile layer. From ancient times and continuing in the present day, people have suffered through varying degrees of heat, redness, pain, swelling and/or stiffness of the joints, any one or all of which can often be associated with deterioration of the articular cartilage in the joints, whether those joints are associated with walking, such as the hip, knee or ankle joints or others, such as the vertebra of the spine, the shoulder, elbow or wrist joints and fingers. Regardless, damage to and/or the deterioration of articular cartilage in a joint is often, if not always accompanied by inflammation. Inflammation, which is typically thought of as heat, redness, pain, swelling and/or stiffness, when experienced in a joint, can be crippling.

[0003] As a result, many have tried to develop ways to alleviate the pain and inflammation associated with arthritis and other damage to the joints. A number of these efforts have focused on oral medications such as cortisone derivatives (steroids) and numerous non-steroidal anti-inflammatory drugs (NSAIDs), all of which have potentially serious side effects. Other efforts have focused on implants of entire joints, such as the knee or hip, although typically, a lengthy and complicated surgical procedure is required, with the patient being forced to undergo a significant recovery period, including a rigorous and costly regimen of physical therapy thereafter. Most often, full motion and full activity are not achieved with the use of these implants. While medical science has recently developed a variety of new materials for the joint implants, these implants are often more costly, offer results which may be only marginally better than those obtained previously, and do nothing to avoid the hospitalization required for the surgical implantation of them nor the long periods of rehabilitation. In addition, it is also possible that one or more revision surgeries will be needed to replace defective, loose or infected implants. Further, the general discomfort which might be associated with utilizing such implants makes an alternative method all the more desirable.

[0004] The biological action of growth hormone, namely, somatotropin, has been the subject of the inventor's research. Heretofore, growth hormone has been used clinically to enhance the growth of children with short stature. Somatotropin may have other effects on other organ systems but in the instant application for a patent, the specific actions of somatotropin related to its effects on articular cartilage have been focused on by the inventor's research and are utilized herein. The major targets of somatotropin activity are believed by the inventor hereof to be vascular sinusoids and subchondral vessels located at the cartilage-bone interface (sub-chondral bone) and the endothelial cells located therein, and in addition, nests of stem (pleuripotential) cells in various sites such as marrow; and the vascular system. More specifically, it is believed by the inventor hereof that growth hormone has the ability to stimulate proliferation of stem cells in the marrow and subchondral vessels and sinusoids. The inventor hereof has also shown that growth hormone has the ability to form vascular and multi-lumen sinusoids, known as Glomeruloids, from pre-existing and mature single lumen vessels in the sub-chondral bone. The inventor describes this action of growth hormone as Morphogenic Action, which is a type of rejuvenation of mature monolumen vessels into fetal-like and/or other immature chondrogenic vascular structures. This Morphogenic Action, a type of rejuvenation, can also dematurate a layer of mature subchondral bone into a cartilaginous state comparable to that observed in the neonatal and immature cartilaginous skeleton.

[0005] The method of this invention relies on a novel use of growth hormone, namely, somatotropin. More in particular, the method of the present invention is useful as an anti-inflammatory agent and is specifically adapted to treat inflammation (heat, redness, pain, swelling, stiffness, etc.) and/or pain associated with damaged and/or defective articular cartilage on or at a joint in a body through the injection directly into the joint of one or more dosages of purified growth hormone (somatotropin). There is no reliance on the transplantation of tissue and thus all of the detrimental conditions of rejection, immune reaction, and other causes of transplant failure are avoided. Similarly, the present invention does not require an individual to undergo a lengthy or complicated surgical procedure, such as those which accompany joint replacements.

[0006] Until the present invention, growth hormone has not, to the inventor's knowledge, ever been used to treat merely the inflammation of tissues such as the soft

tissue components within and surrounding a joint, i.e., synovial lining, capsule, and ligaments and articular cartilage and/or the pain associated therewith. Of course, the inventor herein has heretofore focused on growth hormone as a means for regenerating articular cartilage in a joint, for which U.S. Patent No. 5,368,051 was awarded, incorporated herein by reference, but he has since improved and refined the applications for which growth hormone may be used, as set forth in greater detail, below.

[0007] Accordingly, the method of the present invention provides a much needed improvement in the treatment and elimination of ailments associated with the deterioration or destruction of the articular cartilage surface of a joint, including pain, inflammation of the soft tissue components within and surrounding the joint, including heat, redness, pain, swelling or stiffness. The method of the present invention also is directed towards providing for the reappearance or increase of space between bone ends and restoration of normal alignment of a limb, such as a leg, and including the restoration of normal or nearly normal motion. Further, the present invention is additionally directed towards a preliminary step involving treating a joint with a group of agents such as anti-cytokines, anti-kinases, anti growth factors - used individually or in various combinations thereof, so as to increase the chances that subsequent treatment of the joint for inflammation will be successful.

SUMMARY OF THE INVENTION

[0008] The present invention is directed towards a method of treating inflammation and pain in a joint separating two or more bones. It is pointed out that for purposes of this application, inflammation means pain, joint stiffness, redness, heat and/or swelling, etc.

[0009] The present invention is additionally directed towards a preliminary step involving treatment of the joint with a group of agents such as anti-cytokines, anti-kinases, anti growth factors - used individually or in various combinations thereof - so as to reduce or remove deleterious activity in the joint such that subsequent treatment of the joint for inflammation will likely be successful. More in particular, the preliminary step involves the injection or other application of a group of agents such as anti-cytokines, anti-kinases, anti growth factors - used individually or in various combinations thereof - to a joint about to undergo treatment for inflammation, as described herein.

[0010] Thus, the invention moreover relates to one or more anti-cytokines, anti-kinases and/or anti growth factors, for simultaneous, simultaneous separate or sequential use in the preparation of a joint for anti-inflammation treatment.

[0011] The method comprises an initial step of dissolving a quantity of growth hormone in a buffer solution and then injecting the resulting mixture as a single loading dose into the joint cavity where it will lessen the inflammation of the synovial lining, joint capsule, ligaments and articular cartilage. If desired or needed, additional injections of growth hormone of a single dosage can be injected from one day to several weeks later and after a first set of single or multiple injections, several additional sets of single or multiple injections may be given so as to maintain any improvement of the function of the joint.

[0012] In one alternative embodiment, the method of the present invention may comprise an additional step of mixing an amount of Lidocaine, anywhere from about 0.5 milliliter to 10 milliliters, and ideally about 1 to 3 milliliters of Lidocaine with the mixture of growth hormone and buffer solution. It is contemplated that other injectable anesthetics aside from Lidocaine might also be used with the present invention.

[0013] It is a primary object of the present invention to provide a method for reducing the inflammation of tissue located in or at the joints of a body as well as pain arising at or within the joint of a body without requiring a surgical procedure.

[0014] It is also a primary object of the present invention to provide a new treatment for pain and inflammation in the joint of a body which relies upon a lower dosage of growth hormone and an alternative buffer solution other than that described previously in the applicant's U.S. Patent No. 5,368,051 directed to regenerating articular cartilage.

[0015] A feature thought to arise following treatment of a joint with the present invention is that contact or near contact between the bone-to-bone surfaces is reversed, meaning that a separation, distance or space between the bony surfaces is restored, presumably but perhaps not exclusively because the treatment causes some resumption of growth of articular cartilage, such as that which has been worn down.

[0016] An advantage of the present invention over that disclosed in the Applicant's previous patent is that a range of motion is restored to a joint following treatment.

[0017] Another advantage of the present inventive treatment is the smoothing of irregular joint surfaces and sub-chondral bone and also a reversal of malalignment of the limb following treatment. The present invention thereby eliminates or substantially alleviates ailments in the joints.

[0018] These and other objects, features and advantages of the present invention will become more clear when the drawings as well as the detailed description are taken into consideration.

BRIEF DESCRIPTION OF THE DRAWINGS

[0019] For a fuller understanding of the nature of the present invention, reference should be made to the following detai-led description taken in connection with the accompanying drawings in which:

Figure 1 is a cross-sectional view of a joint surface illustrating a deteriorated articular cartilage on the lower joint surface.

Figure 2 is an isolated view illustrating the injection of a growth hormone and buffer solution in the joint cavity.

[0020] Like reference numerals refer to like parts throughout the several views of the drawings.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

[0021] The present invention is directed specifically towards a method of treating inflammation and associated pain in a joint, such as one having damaged or defective articular cartilage 10. Articular cartilage 10, which is present between bones 15 at a joint 20, provides a bearing type surface for facilitated movement between the bones 15. If articular cartilage is damaged or deteriorated, as represented by reference numeral 30 in the drawings, this can result in a person's experiencing significant heat, redness, pain, swelling, stiffness and/or malalignment of the limb or joint, and can even be crippling to some individuals, such as those suffering from a trauma or other ailments which destroy the joint surface. The articular cartilage 10 is a resilient layer of tissue which covers the ends of bones 15, and it has been traditionally-assumed that once gone, it cannot be regrown or regenerated, at least until the work by the inventor hereof, some of which has been set forth in U. S. Pat. No. 5,368,051.

[0022] The method of the present invention is a significant improvement over what is known in the art for treating the sometimes excruciating pain which individuals experience in one or more of the joints of their bodies. For example, the present invention does not involve a surgical procedure, which would require some recovery therefrom, nor any type of transplantation of tissue. The method of the present invention, which is believed to offer swift relief to the heat, redness, pain, swelling, stiffness or other inflammatory symptoms experienced by individuals suffering from damaged articular cartilage in a joint, offers an improvement over the method described in the inventor's previous U.S. Pat. No. 5,368,051 by relying upon the utilization of a lower dosage of growth hormone and of an alternative buffer solution, and if desired, the addition of injectable anesthetics. The method of the present invention is thought to be effective as a result of the discovery that in addition to the metaphyseal growth plate which exists near the ends of bones and which makes the bones grow during the immature and adolescent periods, there is also an articular growth plate at the joint surface. The metaphyseal growth plate, once achieving full growth within the bone, ceases to function in an adult and disappears. The articular growth plate, however, remains intact, although growth-inactive, at the joint surface in the adult. When properly stimulated by injecting purified growth hormones in the joint, including an anesthetic if desired, as in the method(s) of the present invention, there would be no need for surgically exposing the joint nor for debriding it; the pain and inflammation associated with the damaged articular cartilage is relieved, and this is thought to be because the articular growth plate is stimulated so as to resume active growth.

[0023] With reference now to Figure 1, when an articular cartilage defect as at 30 is present in the joint of an individual, whether a hip joint, knee joint, ankle joint or other type of joint, such that it causes him or her sufficient pain to seek out medical treatment, it is preferable that the individual be required to undergo certain tests in an effort to determine whether treatment in accordance with the present invention is advisable. For example, it is preferred that the individual undergo a complete physical examination by a licensed physician, including any X-rays, MRIs, and/or other laboratory work that may be recommended to hopefully rule out the presence of serious, acute or chronic illnesses and/or whether the individual has a pre-existing excess amount of growth hormone. That is because it is preferred that such persons would not be treated in accordance with the present invention.

[0024] Turning more specifically to the method of the present invention, it is directed preferably for use on humans; however, it can be similarly effective with other animals so long as the necessary growth hormone, preferably purified growth hormone, is utilized. It is preferred that the growth hormone be species specific which means that human growth hormone would be used on humans; cattle (bovine) growth hormone would be used on cattle; and horse growth hormone would be used on horses, etc. More in particular, it is preferred that the growth hormone (known as somatotropin) utilized be identical to naturally produced growth hormones of that species. If a biologically engineered hormone alternative were to be used, it should have an amino acid sequence identical to the natural hormone. In the most preferred embodiments, the growth hormone is biologically engineered to exactly duplicate the natural hormone and to assure maximum purity, and avoid the possibility of transmitting disease. For example, if the growth hormone is to be prepared from pituitary glands retrieved from cadavers, the hormone preparation may transmit rare forms of neurological disease even though it may be highly purified.

[0025] More in particular, the method of the present invention generally comprises the steps of dissolving a quantity of growth hormone, preferably somatotropin that has ideally been biologically engineered so as to be in a purified state, in a buffer solution and then injecting the resulting solution into the joint having damage which causes an individual to experience pain or inflammation. The quantity of growth hormone to be dissolved in the buffer solution is discussed in greater detail below. The purified growth hormone is typically in the form of a pow-

der and as such, may be readily dissolved in a buffer solution. Preferably, the buffer solution has a range of pH between 5.5 and 8.3, although more preferably, the range of pH is between 6.0 and 8.0. Generally, buffer solutions include a saline solution and have a pH range of approximately 7.0 to 7.4 which is the range of biological pH. In a preferred embodiment, the buffer solution comprises a phosphate buffer which may also include a preservative. In an alternative embodiment, the buffer solution is Hank's Buffer Solution having a higher pH range of about 8.0. Other preparations of purified growth hormone may, due to their chemical composition, require buffer solutions of other ranges of pH.

[0026] The growth hormone to be dissolved in the buffer solution can be in a range of between 0.5 milligrams and 10.0 milligrams of growth hormone per milliliter of buffer solution, although a most preferred dosage of about 5.0 to 7.0 milligrams growth hormone, and ideally, 5.8 milligrams of growth hormone per milliliter of buffer solution would be used. This dosage is thought to be operative in accordance with the present inventive method for most human individuals. An alternative dosage to be administered can be more closely related to the person's and/or animal's weight, and will be in the preferred range of 0.025 milligrams to 0.249 milligrams per Kilogram of body weight.

[0027] Once the growth hormone and buffer solution have been mixed, a single dosage of the mixture is injected to the joint, as illustrated in Figure 2. The growth hormone is injected, such as by utilizing a syringe 70, into the joint space and not directly into the bone 15 or tissue. In this manner, it may flow over the entire joint surface and react initially with the tissues on the surface and then with all the vascular units 40 at the bone-cartilage interface. A portion of the purified growth hormone may be absorbed into the bloodstream after about four hours. One of the systemic effects associated with this absorption into the general circulation will be to stimulate production of stem cells in the marrow, vascular system and other areas outside the joint. The growth hormone will cause a reaction in the subchondral vascular structures so as to promote local production of endothelial derived stem cells and also to attract pleuripotential cells to the sinusoidal layer of the bone, the pleuripotential cells being collected in these vascular structures. The reaction will initiate cell layer growth at the subchondral layer, and it is believed will eventually produce enough cartilage to form additional joint surface and lead to there being an increased space between the bones of the joint being treated in accordance with the present invention. Depending on the individual patient's condition, repeated, periodical injections of the growth hormone may be required. For example, another single dosage may be injected into the joint in about four weeks, and repeated in another four weeks. Injections could be given and repeated at other time intervals, however, such as every two weeks. Alternatively, single or multiple injections can be given one day, several days, to several weeks or months apart. Such repeated injections of somatotropin or growth hormone may be necessary in situations where a patient suffers from a disease which will continuously impair or destroy the cartilage surface, or antagonize the action of the growth hormone. It is further contemplated that the injection of growth hormone according to the present invention could include the addition of chemical substances which will block or impede the antagonistic action of proteases, present in certain diseases, that might impair or prevent the beneficial action of the growth hormone within the joint.

[0028] In an alternative embodiment, the method of the present invention may comprise an additional step of mixing Lidocaine or another local anesthetic with the mixture of growth hormone and buffer solution prior to injection into the joint. In this embodiment, the amount of Lidocaine or other anesthetic to be mixed with the growth hormone and buffer solution may be anywhere from 0.5 milliliters to 10 milliliters, although preferably, about 1 to 3 milliliters will be used.

[0029] From the preceding, it is recognized that the present invention may also be considered to include a beneficial anti-inflammatory composition and/or an analgesic composition, both of which may, of course, be utilized within the previously defined methods. Specifically, the anti-inflammatory and/or analgesic composition may comprise a purified growth hormone of between 0.025 milligrams to 0.249 milligrams per kilo of a patient's body weight dissolved in a buffer solution of approximately between 1 to 10 milliliters, preferably as described with regard to the method of treatment, or a purified growth hormone of approximately between 0.5 milligrams to 10.0 milligrams per milliliter of the buffer solution, also preferably as previously recited. Further, a local anesthetic agent, anti-protease agent and/or anti-enzyme agent may be included therewith. In the case of the local anesthetic, it may preferably include Lidocain in an amount of generally between about 0.5 milliliter to 10 milliliters.

[0030] In yet another embodiment, the present invention additionally comprises a preliminary step involving treatment of the joint with an anti-growth factor. More in particular, the preliminary step involves the injection or other application of a group of agents such as anti-cytokines, anti-kinases, anti growth factors - used individually or in various combinations thereof - to a joint about to undergo -treatment for inflammation, as described previously herein. In one embodiment the injection of such agents may be made simultaneously, as in all in one step, or substantially simultaneously, with the injection of growth hormone solution pursuant to the inflammation treatment. For example, the agent(s) may be admixed with growth hormone for the purposes of the present invention. Preferably, the agent is Embrel, a commercially available anti-TNF antibody. The agent is advantageously administered in an amount of about 3mg to about 25mg, in combination with growth hor-

mone, for example by injection into the joint.

[0031] Alternatively, the joint may be preliminarily treated by making one or more intra-articular injections of such agents. Suitable agents include Embrel in a quantity of about 3 to about 25 mg from 1 to 7 days prior to subsequent treatment with growth hormone for inflammation as described herein. The injection may be repeated from 1 to about 4 times, or more. The presence of Embrei or other acents such as anti~cytokines, antikinases, anti growth factors - used individually or in various combinations thereof - has the effect of quieting the joint. This is believed to be due to the reduction and/or removal of the irritating activity of certain agents, e.g. tumor necrosis factor, which might otherwise impede and/or interfere with the responsiveness of the joint to subsequent treatment with growth hormone for inflammation. As such, the preliminary treatment with a group of agents such as anti~cytokines, anti-kinases, anti growth factors - used individually or in various combinations thereof - should aid in the overall success rate of the treatment with the present invention, particularly in patients having rheumatoid joints and/or rheumatoid arthritis.

[0032] In yet another embodiment, the present invention may additionally comprise the use of a lubricant, optionally added to the growth hormone solution. In particular, a lubricant such as purified hyaluronic acid or a hyaluronate salt may be used. Preferably, about 1mg to about 30mg of sodium hyaluronate is administered, about 1 to about 7 days prior to treatment of the joint with growth hormone for inflammation. Alternatively, or in addition, the lubricant may be administered in combination with and/or simultaneously with the growth hormone solution. Furthermore, a mixture of lubricant with growth hormone may be prepared beforehand, and the mixture administered. Advantageously, the lubricant and/or mixture is injected directly into the joint. The administration may be repeated from one to about four times, or more.

[0033] Since many modifications, variations and changes in detail can be made to the described preferred embodiment of the invention, it is intended that all matters in the foregoing description and shown in the accompanying drawings be interpreted as illustrative and not in a limiting sense. As examples, the present invention is also claimed in terms of a method for increasing a patient's range of motion in a joint as well as reducing the mal-alignment of a patient's arthritic joint, the latter of which can be characterized as a bow-legged deformity when the joint involved is the knee. In other words, it is the inventor's belief that the intra-articular injection(s) of growth hormone into joint(s) restores normal alignment of osteo-arthritic and post traumatic arthritic knees, such that a bow leg deformity may disappear and the leg can regain normal alignment, and further, or alternatively, that it can restore normal or nearly normal motion in both extension and flexion in osteoarthritic and post traumatic arthritic knees or other joints. This increased range of motion can be assisted by therapeutic exercise(s), which normally, without treatment in accordance with the present invention, would be extremely painful. In many cases then, therapeutic exercises can only be carried out following treatment with the present invention in as much as the present invention reduces the pain experienced by the patient so as to permit the exercise(s) to occur at all. As another example, the inventor believes that the method of the present invention can be used to treat and/or increase the joint soaces between the vertebrae of the spine, as well. Thus, the scope of the invention should be determined by the appended claims and their legal equivalents.

Claims

1. Use of growth hormone in the manufacture of a composition for treating inflammation in a joint of a body.

2. Use according to claim 1, wherein treating the joint for inflammation comprises the steps of:

   a) dissolving a quantity of purified growth hormone in a buffer solution, and
   b) injecting a single dosage of said growth hormone and said buffer solution into said joint along the joint surface.

3. Use according to claim 2, wherein said buffer solution has a range of pH between generally about 5.5 and 8.3.

4. Use according to claim 3, wherein said buffer solution is a phosphate buffer solution.

5. Use according to claim 3, wherein said buffer solution is Hank's Buffer Solution having a pH of about 8.0 to 8.3.

6. Use according to any preceding claim wherein said growth hormone is species specific so as to be identical to naturally produced growth hormones.

7. Use according to any preceding claim wherein said growth hormone is biologically engineered to assure maximum purity and disease elimination.

8. Use according to any one of claims 2 to 7 wherein a range of 0.5 to 10.0 milligrams of said purified growth hormone is dissolved in generally about 1 to 10 milliliters of said buffer solution, the total volume of said dosage being dependent upon the weight of the individual subject being injected, and the size of the joint.

9. Use according to claim 8 wherein said dosage is a single dosage and is between generally about 0.025 to 0.249 milligrams of purified growth hormone per Kilogram of body weight.

10. Use according to claim 9 wherein about 5.8 milligrams of said purified growth hormone is dissolved in 1 to 10 milliliters of said buffer solution.

11. Use according to any one of claims 8 to 10 further comprising the step of injecting a second one of said single dosage into the joint generally about one week later.

12. Use according to claim 11, further comprising the steps of injecting a third one of said single dosage into the joint generally about one week later.

13. A Use according to any one of claims 8 to 10, further comprising the steps of injecting a second one of said single dosage into the joint generally about two weeks later.

14. Use according to claim 13, further comprising the steps of injecting a third one of said single dosage into the joint generally about two weeks later.

15. Use according to any one of claims 8 to 10, further comprising the step of injecting a second one of said single dosage into the joint generally about four weeks later.

16. Use according to claim 15, further comprising the steps of injecting a third one of said single dosage into the joint generally about four weeks later.

17. Use according to any preceding claim, further comprising the step of mixing generally about 0.5 milliliters to 10 milliliters of a local anesthetic with said mixture of growth hormone and buffer solution.

18. Use according to claim 17 wherein the local anesthetic is Lidocaine.

19. Use of a single dosage of a growth hormone in a range of 0.025 milligrams to 0.249 milligrams per kilogram of patient body weight dissolved in a buffer solution in the manufacture of a composition for increasing a patient's range of motion of a joint.

20. Use of at least a single dosage of a growth hormone in a range of 0.025 milligrams to 0.249 milligrams per kilogram of patient body weight dissolved in a buffer solution in the manufacture of a composition for correcting a malalignment in an arthritic joint of a body, such as a bow-legged deformity.

21. Use of at least a single dosage of a growth hormone in a range of 0.025 milligrams to 0.249 milligrams per kilogram of patient body weight dissolved in a buffer solution in the manufacture of a composition for increasing the space between the bone ends of a patient's joint.

22. Use of at least a single dosage of a growth hormone in a range of 0.025 milligrams to 0.249 milligrams per kilogram of patient body weight dissolved in a buffer solution in the manufacture of a composition for smoothing the surface of the bone ends of a patient's joint.

23. An anti-inflammatory composition comprising a purified growth hormone of between 0.025 milligrams to 0.249 milligrams per kilo of a patient's body weight dissolved in a buffer solution of between 1 to 10 milliliters.

24. An anti-inflammatory composition as recited in claim 23 further comprising a local anesthetic.

25. An anti-inflammatory composition as recited in claim 23 further comprising an anti-protease agent.

26. An anti-inflammatory composition as recited in claim 23 further comprising an anti-enzyme agent.

27. An anti-inflammatory composition comprising a purified growth hormone of between 0.5 milligrams to 10.0 milligrams per milliliter of a buffer solution.

28. An anti-inflammatory composition as recited in claim 27 further comprising a local anesthetic.

29. An anti-inflammatory composition as recited in claim 27 further comprising an anti-protease agent.

30. An anti-inflammatory composition as recited in claim 27 further comprising an anti-enzyme agent.

31. An analgesic composition comprising a purified growth hormone of between 0.025 milligrams to 0.249 milligrams per kilo of a patient's body weight dissolved in a buffer solution of between 1 to 10 milliliters.

32. An analgesic composition as recited in claim 31 further comprising a local anesthetic.

33. An analgesic composition as recited in claim 32 wherein said local anesthetic comprises Lidocain in an amount of generally between about 0.5 milliliter to 10 milliliters.

34. An analgesic composition as recited in claim 33 further comprising a local anesthetic agent.

35. An analgesic composition as recited in claim 33 further comprising an anti-protease agent.

36. An analgesic composition as recited in claim 33 further comprising an anti-enzyme agent.

37. An analgesic composition as recited in claim 31 further comprising an anti-protease agent.

38. An analgesic composition as recited in claim 31 further comprising an anti-enzyme agent.

39. An analgesic composition comprising a purified growth hormone of between 0.5 milligrams to 10.0 milligrams per milliliter of a buffer solution.

40. Use according to any one of claims 1 to 22, further comprising the administration of a group of agents such as anti-cytokines, anti-kinases, anti growth factors - used individually or in various combinations thereof.

41. Use according to claim 40, wherein one or more agents is administered in combination with the growth hormone.

42. Use according to claim 40, wherein one or more agents is administered prior to treatment with growth hormone for inflammation.

43. Use according to any one of claims 1 to 22, or 40 to 42, further comprising the use of a joint lubricant.

44. Use according to claim 43, wherein the lubricant is sodium hyaluronate.

45. Growth hormone and a group of agents such as anti-cytokines, anti-kinases and anti growth factors, for simultaneous, simultaneous separate or sequential use in the treatment of inflammation of a joint.

46. Growth hormone, a joint lubricant and a group of agents such as anti-cytokines, anti-kinases and anti growth factors, for simultaneous, simultaneous separate or sequential use in the treatment of inflammation of a joint.

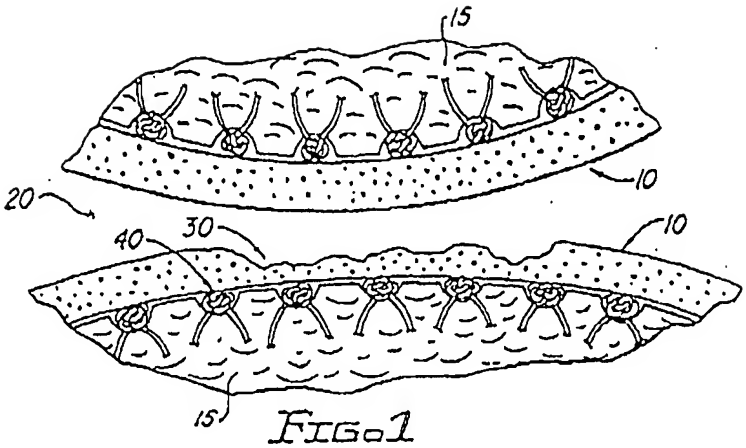

FIG. 1

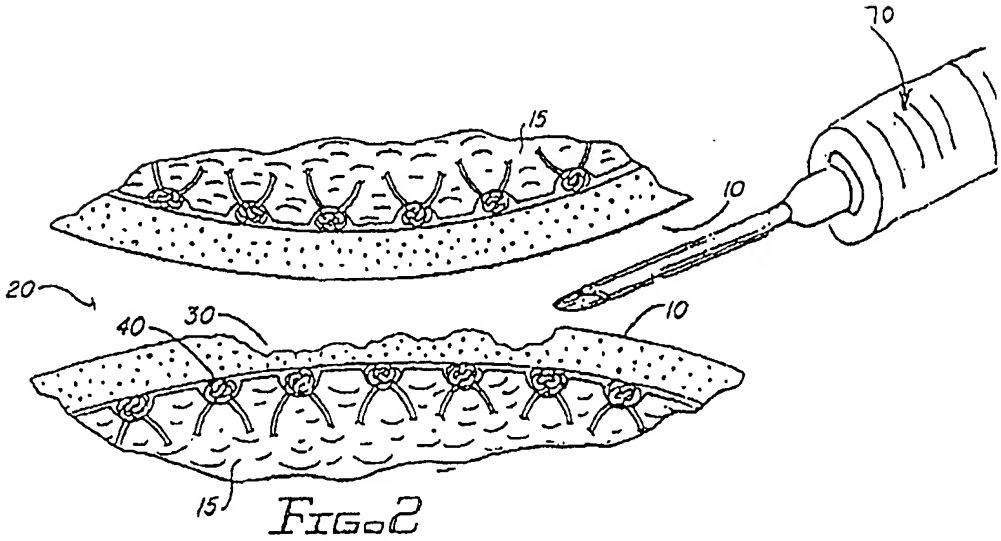

FIG. 2

REVIEW ARTICLE

Sports Med 2003; 33 (5): 381-394
0112-1642/03/0005-0381/$30.00/0

The Roles of Growth Factors in Tendon and Ligament Healing

Timothy Molloy, Yao Wang and *George A.C. Murrell*

Orthopaedic Research Institute, St George Hospital Campus, University of New South Wales, Sydney, NSW, Australia

Contents

Abstract 381
1. Characterisational Studies 383
   1.1 Insulin-Like Growth Factor-I (IGF-I) 383
   1.2 Transforming Growth Factor β (TGFβ) 385
   1.3 Vascular Endothelial Growth Factor (VEGF) 386
   1.4 Platelet-Derived Growth Factor (PDGF) 387
   1.5 Basic Fibroblast Growth Factor (bFGF) 387
2. *In Vivo* Studies 388
   2.1 IGF-1 388
   2.2 TGFβ 389
   2.3 PDGF 389
   2.4 bFGF 390
3. Future Directions 391
4. Conclusion 392

Abstract

Tendon healing is a complex and highly-regulated process that is initiated, sustained and eventually terminated by a large number and variety of molecules. Growth factors represent one of the most important of the molecular families involved in healing, and a considerable number of studies have been undertaken in an effort to elucidate their many functions. This review covers some of the recent investigations into the roles of five growth factors whose activities have been best characterised during tendon healing: insulin-like growth factor-I (IGF-I), transforming growth factor β (TGFβ), vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), and basic fibroblast growth factor (bFGF). All five are markedly up-regulated following tendon injury and are active at multiple stages of the healing process. IGF-I has been shown to be highly expressed during the early inflammatory phase in a number of animal tendon healing models, and appears to aid in the proliferation and migration of fibroblasts and to subsequently increase collagen production. TGFβ is also active during inflammation, and has a variety of effects including the regulation of cellular migration and proliferation, and fibronectin binding interactions. VEGF is produced at its highest levels only after the inflammatory phase, at which time it is a powerful stimulator of angiogenesis. PDGF is produced shortly after tendon

damage and helps to stimulate the production of other growth factors, including IGF-I, and has roles in tissue remodelling.

In vitro and *in vivo* studies have shown that bFGF is both a powerful stimulator of angiogenesis and a regulator of cellular migration and proliferation. This review also covers some of the most recent studies into the use of these molecules as therapeutic agents to increase the efficacy and efficiency of tendon and ligament healing. Studies into the effects of the exogenous application of TGFβ, IGF-I, PDGF and bFGF into the wound site singly and in combination have shown promise, significantly decreasing a number of parameters used to define the functional deficit of a healing tendon. Application of IGF-I has been shown to increase in the Achilles Functional Index and the breaking energy of injured rat tendon. TGFβ and PDGF have been shown separately to increase the breaking energy of healing tendon. Finally, application of bFGF has been shown to promote cellular proliferation and collagen synthesis *in vivo*.

Tendons are the connective tissue that attach muscle to bone, and allow the transduction of force of a contracting muscle to be exerted via the attached skeletal structure.[1] They consist primarily of water and type I collagen, with smaller amounts of other collagens and matrix materials, and various types of cells, most notably fibroblasts.

The process of tendon healing represents an interesting paradigm for medical science. Although most tendons have the ability to heal spontaneously after injury, the scar tissue that is formed is almost always mechanically inferior and therefore much less able to perform the functions of a normal tendon, and is also more susceptible to further damage.[2] Because the formulation of effective treatments for tendon injuries based on traditional tissue-level reparative procedures, surgical or otherwise, has presented such a problem to clinicians in the field, much research has been directed toward the understanding of the mechanisms of tendon healing at the molecular level. This has ultimately been in an effort to develop therapies to facilitate tendon healing through the use of individual molecules or groups of molecules known to have beneficial roles in the process.

The process of tendon healing follows a pattern similar to that of other healing tissues (table I).[2] Upon tissue damage, blood vessels will rupture and signalling molecules released by intrinsic cells will trigger a coagulation cascade that will coordinate the formation of a clot around the injured area. The clot will contain cells and platelets that will immediately begin to release a variety of molecules, most notably growth factors (such as platelet-derived growth factor [PDGF], transforming growth factor β [TGFβ], and insulin-like growth factor [IGF]-I and -II), causing acute local inflammation. During this inflammatory phase, there is an invasion by extrinsic cells such as neutrophils and macrophages which clean up necrotic debris by phagocytosis, and together with intrinsic cells (such as endotenon and epitenon cells) produce a second battery of cytokines to initiate the reparative phase. This stage sees collagen deposition and granulation tissue formation, as well as neovascularisation, extrinsic fibroblast migration and intrinsic fibroblast proliferation. These fibroblasts are responsible for synthesising the new extracellular matrix, consisting largely of collagens and glycosaminoglycan. Finally, a remodelling phase begins, which sees decreases in the cellular and vascular content of the callus tissue, and increases in collagen type I content and density. Eventually, the collagen will become more organised and is orientated and cross-linked with the healthy matrix outside the injury area. After the healing process is complete, cellularity, vascularity, and collagen makeup will return to something approximating that of the normal tendon, although the diameters and cross-linking of the collagen fibrils often remain inferior after healing.[3] This mechanically inferior

Table I. Summary of the healing process in tendons and ligaments

Time (days)	Phase	Process
0	Immediately post-injury	Clot formation around the wound
0–1	Inflammatory	First battery of growth factors and inflammatory molecules produced by cells within the blood clot
1–2	Inflammatory	Invasion by extrinsic cells, phagocytosis
2–4	Proliferation	Further invasion by extrinsic cells, followed by a second battery of growth factors that stimulate fibroblast proliferation
4–7	Reparative	Collagen deposition; granulation tissue formation; revascularisation
7–14	Reparative	Injury site becomes more organised; extracellular matrix is produced in large amounts
14–21	Remodelling	Decreases in cellular and vascular content; increases in collagen type I
21+	Remodelling	Collagen continues to become more organised and cross-linked with healthy matrix outside the injury area. Collagen ratios, water content and cellularity begin to approach normal levels

repair tissue is weaker and more susceptible to tendon creep than uninjured tendon, and is therefore at higher risk of further damage.

The description of tendon healing above is somewhat generalised, and it is important to note that there are slight differences in the way different tendons heal, for example intrasynovial versus extrasynovial tendons. Whereas extrasynovial tendons can be easily influenced by growth factors and cytokines produced by extrinsic cells, for example from the paratenon, intrasynovial tendons are more reliant on intrinsic cells such as those derived from the epitenon and endotenon. These differences are most probably due to differences in the local environment and the ease with which needed growth factors can be provided to the injured area.[4] Although cells that originate from different regions of the tendon can have somewhat different influences during tissue repair, for most types of tendon both extrinsic and intrinsic cells will contribute to healing.[5]

Growth factors have a number of crucial roles in tendon healing. Growth factors such as TGFβ, IGF-I, PDGF, vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) are markedly up-regulated throughout tendon repair. They can potentially be produced by both intrinsic (for example epitenon) and extrinsic (for example macrophage) cells, often have dose-dependent effects, require specific receptors to be active, and usually work in synergy with other signalling molecules. Almost all are up-regulated when transcriptional factors (such as early growth response-1, which stimulates the production of acidic fibroblast growth factor, bFGF, TGFβ, PDGF, hepatocyte growth factor, VEGF and IGF-II, among others[6]), bind to their (often common) regulatory sites.[7] Whilst a large amount of data on these molecules have been produced in recent years, much work still needs to be undertaken to fully understand their varied functions and multiple synergies. This review will cover some of the more recent studies on the functions and clinical applications of five of the best studied growth factors during tendon healing: IGF-I, TGFβ, VEGF, PDGF and bFGF.

1. Characterisational Studies

Growth factors represent one of the largest of the molecular families involved in the healing process, and a considerable number of studies have been undertaken in an effort to elucidate their many functions and behaviours during healing progression (table II). Some of this work, with a focus on IGF-I, TGFβ, VEGF, PDGF, and bFGF, is outlined below.

1.1 Insulin-Like Growth Factor-I (IGF-I)

IGF-I is a single chain polypeptide that shows structural homology to proinsulin, and is involved in both normal body growth and healing.[26] It binds to two types of receptors, type I IGF receptor and type II mannose-6-phosphate receptor,[27] and is regulated by a group of specific IGF binding proteins.[28] It is an important mediator in all phases of wound

Table II. Summary of the roles of five growth factors during tendon and ligament healing

Growth factor	Phase in which growth factor is most active	Roles	Reference
IGF-I	Inflammation, proliferation	Promotes the proliferation and migration of cells, stimulates matrix production	8-13
TGFβ	Inflammation	Regulates cell migration, proteinase expression, fibronectin binding interactions, termination of cell proliferation, and stimulation of collagen production	14-19
VEGF	Proliferation, remodelling	Promotes angiogenesis	20,21
PDGF	Proliferation, remodelling	Regulates protein and DNA synthesis at the injury site, regulates the expression of other growth factors	10,22
bFGF	Protiferation, remodelling	Promotes cellular migration, angiogenesis	23-25

bFGF = basic fibroblast growth factor; **IGF-I** = insulin-like growth factor-I; **PDGF** = platelet-derived growth factor; **TGFβ** = transforming growth factor; **VEGF** = vascular endothelial growth factor.

healing, particularly during the inflammatory and proliferative stages.[8] Injured tissues lacking the growth factor are significantly disadvantaged in healing.[29] Several studies[8,30-34] have shown that IGF-I is locally increased during and after inflammation following soft tissue injury, both at the mRNA and protein levels, and is associated with a corresponding up-regulation of its receptors.[35] Sciore et al.[8] demonstrated that IGF-I mRNA levels were more than 5-fold higher compared with controls 3 weeks after injury to the rabbit medial collateral ligament (MCL), then decreased (yet still remained at levels twice of that of the control) by weeks 6 and 14 (figure 1). Hansson et al.[9] showed that this up-regulation could also be seen at the protein level.

Because IGF-I is such a versatile and widespread signal molecule, it has numerous and varied activities during tendon healing, particularly when working in concert with other growth factors.[10] Its primary roles seem to be to stimulate the proliferation and migration of fibroblasts and other cells at the site of injury, and to subsequently increase the production of collagens and other extracellular matrix structures in these cells during the remodelling stages.[11,12] This proliferative activity was demonstrated by Jones and Clemmons[11] in various cell types, including fibroblasts. The ability of IGF-I to stimulate cells to produce collagen and fibronectin *in vitro* has been shown in rat calvarial cultures.[13]

As with many other cytokines, synergism with other molecules is important for its stimulatory activity. It is thought that IGF-I works to promote cell proliferation when in the presence of other growth factors, such as the PDGF isomer PDGF-BB, discussed in section 1.4. This was shown in *in vitro* work by Tsuzaki et al.[30] in which mitogenesis and subsequent cell division of tendon fibroblasts and tendon surface cells was highest when both growth factors were applied together, compared with their individual application.

It is also interesting to note that Tsuzaki et al.[30] observed that normal avian flexor tendon cells con-

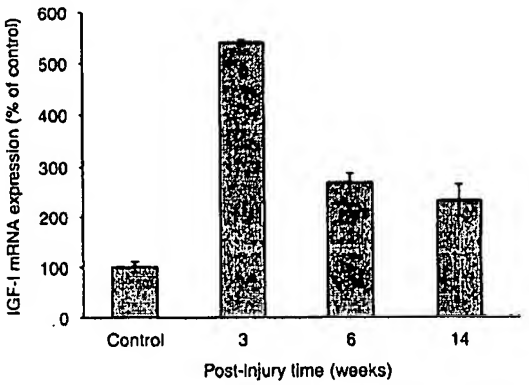

Fig. 1. Semi-quantitative reverse transcription polymerase chain reaction analysis of insulin-like growth factor-I (IGF-I) mRNA expression (expressed as percentage of control) from the injured medial collateral ligament of the New Zealand white rabbit at 3 weeks (n = 4; eight ligaments), 6 weeks (n = 4; eight ligaments), and 14 weeks (n = 4; eight ligaments) post-injury, and uninjured controls (n = 3; six ligaments). All were found to be significantly different from controls by analysis of variance ($p < 0.05$) [reproduced from Sciore et al.,[8] with permission from Elsevier Science].

tained a relatively high abundance of IGF-I protein as quantified by radioimmunoassay, but expression of IGF-I mRNA measured by reverse transcription polymerase chain reaction was very low. This can be explained by the observation that low-level expression of the gene within the normal tendon produces IGF-I protein which is immediately bound by specific binding proteins (such as binding protein-3). These binding proteins keep IGF-I in an inactive form and protect it from degradation. It was suggested that this reservoir of inactivated IGF-I protein is kept extracellularly until tissue injury occurs, at which time enzymes are released that free the bound IGF-I, activating it. This strategy of synthesising a comparatively small number of enzymes to activate a large reservoir of inactive regulatory molecules ensures a rapid response to tissue injury.

1.2 Transforming Growth Factor β (TGFβ)

TGFβ has shown to be active in almost all stages of tendon healing[14] and has such varied effects as stimulating extrinsic cell migration, regulation of proteinases,[15] fibronectin binding interactions,[16] termination of cell proliferation via cyclin-dependent kinase inhibitors[17] and stimulation of collagen production.[18] Three 25-kDa homodimeric mammalian isoforms exist (β1, β2 and β3), and studies in knockout mice have shown that each of these gives rise to a distinct phenotype.[36] They can be produced by most cells involved in the healing process[14] and bind to three distinct classes of membrane receptors, RI, RII and RIII.[37]

TGFβ-1 mRNA expression has been shown to dramatically increase a short time after tendon injury (figure 2) and is thought, in particular, to play an important role in the initial inflammatory response to tissue damage. Studies using lactate, one of the earliest mediators in wound healing due to its rapid build-up during tissue hypoxia, showed that it had the ability to directly stimulate TGFβ-1 production in flexor tendon cells.[15] Natsu-ume et al.[19] demonstrated a significant and early elevation in TGFβ-1 levels in the healing rat patellar ligament which remained high for at least 8 weeks. Immunohistochemical methods showed that initially the

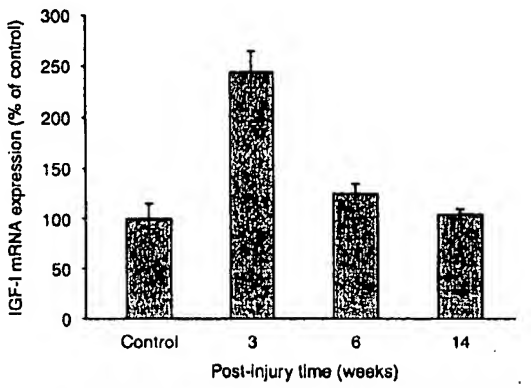

Fig. 2. Semi-quantitative reverse transcription polymerase chain reaction analysis of transforming growth factor β (TGFβ-1) mRNA levels (expressed as percentage of control) from the injured medial collateral ligament of the New Zealand white rabbit at 3 weeks (n = 4; eight ligaments), 6 weeks (n = 4; eight ligaments), and 14 weeks (n = 4; eight ligaments) post-injury, and uninjured controls (n = 3; six ligaments). TGFβ-1 mRNA levels at week 3 only were significantly different from controls by analysis of variance ($p < 0.05$) [reproduced from Sciore et al.,[8] with permission from Elsevier Science].

TGFβ-1 was extracellular, probably due to degranulation by platelets, but later was cell-associated, reflecting *de novo* synthesis.

Klein[38] further showed in work on cultured rabbit sheath, epitenon and endotenon cells that each of the three TGFβ isoforms (TGFβ-1, -2 and -3) has effects on collagen production and cell viability. All three isoforms at two different concentrations (1 and 5 μg/L) decreased the number of cultured cells compared with controls; however, the differences did not reach statistical significance. Production of collagen types I and III (the most abundant types of collagen found in tendon) was significantly increased ($p < 0.05$) in all cell types, although higher growth factor concentration was generally not correlated with further increases in production.

Growth factors lack biological activity unless they bind to their specific receptors, so it follows that TGFβ receptors are also seen to be up-regulated during tendon healing. During healing of transected middle digit flexor digitorum profundus tendons, Ngo et al.[37] used immunohistochemical staining to show up-regulation of all three classes of TGFβ receptor proteins. Levels peaked at postoperative

day 14, and had decreased by day 56. They were abundant along both the tendon sheath and epitenon, suggesting that both intrinsic and extrinsic mechanisms of tendon healing were active in this model.

Similar to other growth factors, TGFβ-1 works in a dose-dependent manner and in synergy with other growth factors.[39] For example, *in vitro* studies on canine anterior cruciate ligament fibroblasts showed that low doses of TGFβ-1 act positively with the PDGF isomer PDGF-AB to promote fibroblast proliferation, whereas at increased concentrations, this was reversed.[20]

1.3 Vascular Endothelial Growth Factor (VEGF)

The growth factors discussed in sections 1.1 and 1.2 become active almost immediately following tissue injury and continue to regulate the function of various processes at almost all phases of healing. However, this early and almost continuous activation is not common to all growth factors. VEGF for example, while having some role in early cellular migration and proliferation, is most active after inflammation, most notably during the proliferative and remodelling phases where it has been shown to be a powerful stimulator of angiogenesis.[40] A number of different isoforms of VEGF exist which appear to have unique biological functions, although all bind to three structurally related receptor tyrosine kinases called VEGF receptor (VEGFR)-1, -2, and -3.[41] The proliferative and mitogenic activities of VEGF chiefly depend on its interactions with VEGFR-2.[42]

Expression of the VEGF gene can be up-regulated in response to both biological and biomechanical stimuli, including hypoxia,[43] other growth factors,[21] interleukins,[43] and, during osteogenesis, bone distraction. Increased levels of angiogenic growth factors such as VEGF within an injury site are correlated with a well-defined pattern of vascular ingrowth from the epi- and intra-tendinous blood supply toward the site of repair. This neovascularisation proceeds along the surface of the epitenon, through a normally avascular area, and provides extrinsic cells, nutrients, and growth factors to the injured area.

Boyer et al.[20] quantified VEGF mRNA levels in the canine intrasynovial flexor tendon at various time points following tendon transection using Northern blot analyses (figure 3). It was found that at days 0 and 4 following injury, levels remained approximately at baseline, which was followed by a peak at day 7 (with levels at approximately 210% that of normal), and then a steady decline back to baseline by day 21. This kind of temporal expression profile is consistent with observed neovascularisation in and around the tendon repair site following inflammation. For example, studies by Gelberman et al.[21] on the canine flexor tendon showed an increase in vessel length and density starting from post-operative day 3, which peaked at day 17, and was followed by a decrease in vessel density at day 28.

Using *in situ* hydridisation on the injured canine flexor tendon, the spatial pattern of VEGF expression has also been determined.[40,44] VEGF mRNA accumulation was detected in around 67% of cells at the injury site, whereas only 10% of the epitenon cells directly adjacent showed accumulation, and had levels comparable to those epitenon cells distant from the site of repair. This extremely apparent stratification of gene expression between adjacent

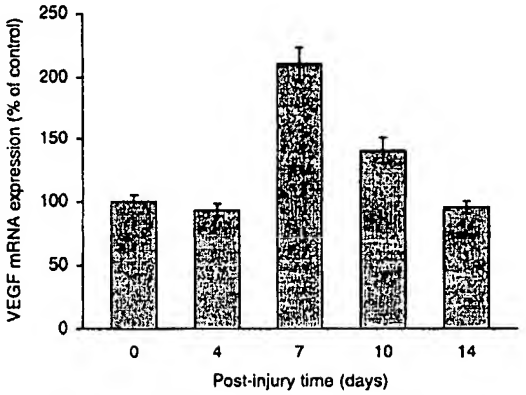

Fig. 3. Northern Blot analyses of vascular endothelial growth factor (VEGF) mRNA expression in the canine flexor tendon post injury, compared with controls. A statistically significant elevation in VEGF mRNA was shown at days 7 and 10 post-injury ($p < 0.05$) [reproduced from Boyer et al.,[20] with permission from Elsevier Science].

cells effectively demonstrates how highly coordinated the mechanisms of healing are. This coordination is brought about largely through the specificity of action and tight regulation of growth factors and other molecules during each of the phases of healing.

1.4 Platelet-Derived Growth Factor (PDGF)

PDGF describes a group of dimeric polypeptide isoforms made up from three types of structurally similar subunits. Its activity is mediated through its interaction with two related tyrosine kinase receptors, one of which binds all three PDGF chains, and the other binds only one.[45] Work by Duffy et al.[44] has shown that PDGF is elevated in the healing canine digital flexor tendon, suggesting a role in the healing process. It is thought to play a significant role in the early stages of healing, at which time it induces the synthesis of other growth factors, such as IGF-I.[10] *In vitro* studies by Yoshikawa and Abrahamsson[46] on PDGF have further demonstrated that this growth factor also plays an important role during tissue remodelling. PDGF was observed to stimulate both collagen and non-collagen protein production, as well as DNA synthesis, in a dose-dependent manner.

One theory that has been put forward as to how PDGF increases protein production involves its induction of TGFβ-1 expression.[22] However, *in vivo* studies by Hildebrand et al.[47] in which the PDGF isomer PDGF-BB was applied to the healing MCL of the rabbit with and without TGFβ-1 showed no such complementary effect. In fact, addition of PDGF-BB and TGFβ-1 together resulted in poorer healing (as determined by ultimate load, energy absorbed to failure, and ultimate elongation values) than addition of PDGF-BB alone. Stimulation of DNA synthesis by PDGF has also been postulated to occur through a growth factor second messenger. In this case, increases in PDGF have been shown to result in up-regulation of IGF-I and IGF receptors, that once activated stimulate DNA synthesis.[10] A significant amount of the PDGF produced for this end is thought to come from an exogenous source, probably from platelets.[30]

Interestingly, the level of stimulation has been shown to be specific to the site and type of tendon examined. In studies by Yoshikawa and Abrahamsson[46] DNA synthesis was stimulated to higher levels in intermediate compared with intrasynovial tendons, and protein synthesis was higher in proximal intrasynovial tendon segments than in extrasynovial peroneal tendon segments.

1.5 Basic Fibroblast Growth Factor (bFGF)

The final growth factor that will be discussed here is bFGF. It is a single chain polypeptide composed of 146 amino acids, and is a member of the heparin-binding growth factor family.[35] Through its interaction with a number of isoforms of four cell surface receptors,[48] it has been shown to be a potent stimulator of angiogenesis and cellular migration and proliferation in both *in vivo* and *in vitro* studies.[23]

Stimulation of cellular migration and proliferation by bFGF has been demonstrated by Chan et al.[49] using cultured rat patellar tendon fibroblasts. In this study, an '*in vitro* wound' was created by mechanically generating a uniform cell-free zone in a culture dish. The progression of closure of the *in vitro* wound was measured at various time points after the addition of four different concentrations of bFGF, ranging from 0–50 μg/L. It was observed that the addition of as little as 2 μg/L of bFGF accelerated the rate at which wound closure progressed, and a concentration of 10 μg/L was most effective. Cellular proliferation was confirmed as the mechanism of wound closure and distinguished from cell chemotaxis by the measurement of 5-bromo-2'-deoxyuridine incorporation.

A later study by Chang et al.[25] used a rabbit flexor tendon model to localise and quantify bFGF mRNA during tendon healing. *In situ* hybridisation showed that bFGF expression was increased in both the tendon parenchyma and the tendon sheath from the first postoperative day, and remained elevated up to the last time point, day 56. The highest levels of expression within the tendon came from intrinsic tenocytes and fibroblasts migrating from the epitenon, along the edge of the wound. Inflammatory

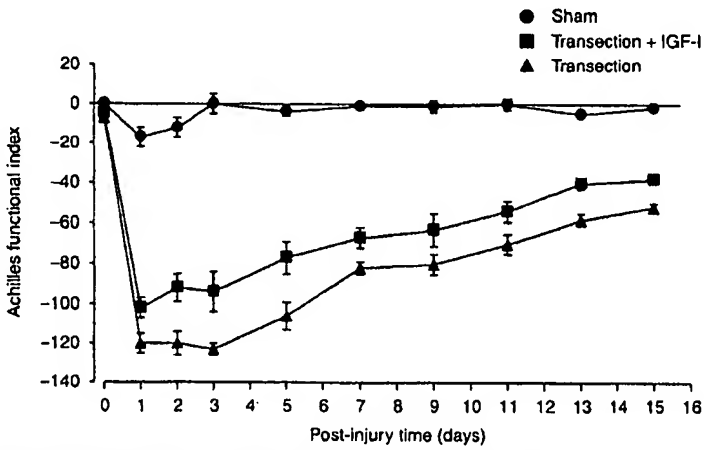

Fig. 4. 15-day time course showing the effects of insulin-like growth factor-I (IGF-I) [± SEM] on the Achilles Functional Index of the rat following tendon transection. Within a given time point all groups are significantly different ($p < 0.05$) [reproduced from Kurtz et al.,[50] with permission].

leucocytes and fibroblasts in the surrounding synovial tendon sheath also displayed high bFGF messenger levels. These observations again suggest that both intrinsic and extrinsic cells are important in tendon healing as sources of growth factors.

2. *In Vivo* Studies

Shortly after the initial investigations which discovered and characterised some of the more important growth factors employed in tendon and ligament healing, clinical studies commenced in a variety of animal models. Because tendon healing is a complex process involving the interaction of a large number of different molecules, cells and tissues, results have often been unpredictable and disappointing. However, some success has been achieved, which suggests that the speed and quality of tendon healing may eventually be improved by the application and/or regulation of growth factors and other molecules. The major challenges seem to be in predicting the synergies and antagonisms among growth factors and between growth factors and other molecules, and how to temporally and spatially apply different growth factors for best effect. Another major technical challenge common to all *in vivo* studies is the delivery of the therapeutic molecules to the target cells in a specific and sustained manner.

In vivo use of TGFβ, IGF-I, PDGF, bFGF, singly and in combination has shown some promise in recent years. The following is a brief summary of the most recent *in vivo* work for each of these growth factors.

2.1 IGF-1

IGF-I has been successfully used by Kurtz et al.[50] to increase the rate of healing in the transacted rat Achilles tendon (figure 4). Following transection, each tendon was treated with 25μg of a recombinant variant form of IGF-I (a form which has much less binding affinity to circulating proteins) in a methylcellulose gel vehicle. An obvious positive effect on the healing tissue was observed as early as 24 hours after the transection and addition of IGF-I (as shown by measurements of the Achilles Functional Index), and this effect continued up until the tenth and last measurement, on day 15.

The same study also showed the ability of IGF-I to reduce inflammation and its resulting functional deficit in damaged tendons. In experiments similar to those described above, 20 rats underwent Achilles tendon transection followed by an injection of the inflammatory agent carrageenan. The injury was

subsequently treated with the recombinant IGF-I, and functional and biomechanical data collected. It was observed that the rats that received IGF-I had a much less functional deficit induced by the carrageenan than rats that did not receive IGF-I. Although the exact mechanism by which IGF-I regulates inflammation is unknown, it was postulated that instead of simply preventing inflammatory cells from migrating into the injury area (as there was no significant decrease of these types of cells in the wound), it may act through a negative feedback loop. As one of the main products of the inflammatory cascade, high concentrations of IGF-I may act to switch off early inflammatory cascade genes in the cells involved in this process.

The ability of IGF-I to augment ligament healing when in combination with bFGF has also been studied. In one study,[51] a small incision in the MCL of the rat was treated with a collagen emulsion/IGF-I/bFGF preparation and left to heal (with no ligament repair) for 12 days, after which the ligament was extracted and its biomechanical properties tested. A statistically significant increase in the breaking energy of 58% (±83%, $p < 0.05$, $n = 10$) was observed; however, measurements of rupture force and stiffness were found not to be significantly different from the controls.

2.2 TGFβ

In vivo studies do not always involve the exogenous application or up-regulation of a particular growth factor; for many growth factors, too high a dose can in fact be detrimental. High levels of TGFβ-1, for example, have been implicated in tendon adhesion formation, which can significantly decrease the range of motion of a tendon.[49] In an effort to counter this, Chang et al.[14] have conducted studies on TGFβ-1 and -2 within the healing rabbit zone II flexor tendon. Their work used neutralising TGFβ-1 and -2 antibodies in an attempt to decrease TGFβ-1 and -2 activity and the associated loss of range of motion. Twenty-two animals underwent a transection of the zone II middle digit flexor digitorum profundus followed by a treatment of either phosphate-buffered saline, TGFβ-1 antibody, or a combination of TGFβ-1 and -2 antibodies. They observed that the animals that received antibodies to TGFβ-1 had around twice the range of motion (defined as the combined angular measurement of flexion at the proximal and distal interphalangeal joints) than those that did not. Interestingly, animals that received antibodies to both TGFβ-1 and -2 did not show a significantly higher range of motion than those that received only TGFβ-1 antibodies.

Other members of the TGFβ superfamily have been used *in vivo* with some success. Forslund and Aspenburg[52,53] used a single direct injection of cartilage-derived morphogenetic protein-2 (CDMP-2; also known as GDF-6 or bone morphogenetic protein-13), into the transected Achilles tendons of rats, and observed an increase in the force at failure of 39% in rats treated with CDMP-2 versus the control. Also, the tendons treated with CDMP-2 were thicker and appeared more dense than the non-treated controls. CDMP-1 was used in an earlier study from the same laboratory, but it seemed less potent as shown by a two-way ANOVA. In a third study, Aspenburg and Forslund[53] used the TGFβ family member osteogenic protein-1, but this was shown to induce bone formation in the tendon and had a detrimental effect on mechanical strength.

2.3 PDGF

Letson and Dahners[51] used treatments of PDGF alone, PDGF in combination with IGF-I, and PDGF in combination with bFGF, in an attempt to improve the healing of the rat MCL. 1.2μg of each growth factor in a collagen emulsion was injected into the transected ligament, and at day 12 post-injury the ligaments were harvested and their biomechanical properties tested. They observed that the PDGF-only treatment increased healed ligament strength by 73% (±55%, $p < 0.0025$), stiffness by 94% (±63%, $p < 0.0025$), and breaking energy by 101% (±104%, $p < 0.01$; not statistically significant). Likewise, the PDGF + IGF-I and PDGF + bFGF treatments also increased the quality of ligament healing to a similar level versus controls; however, in this case no synergistic interactions were observed. It was suggested that this was perhaps due to sub-

Table III. Structural properties of the healing rabbit femur-MCL-tibia complexes after treatment with a high (20μg) or low (0.04μg) dose of PDGF-BB or control. Data in the experimental/sham section are normalised and expressed as experimental divided by sham (reproduced from Hildebrand et al.,[47] with permission)

Property	Fibrin sealant	Low-dose PDGF-BB	High-dose PDGF-BB
Experimental			
Stiffness (N/mm)	22.4 ± 4.6	30.8 ± 2.6	24.4 ± 11.0
Ultimate load (N)	83.7 ± 28.4	119.4 ± 47.6	130.2 ± 86.4
Energy absorbed (J)	125 ± 25	350 ± 120	380 ± 340
Ultimate elongation (mm)	4.0 ± 0.5	4.7 ± 1.9	5.6 ± 2.1
Experimental/sham			
Stiffness (N/mm)	0.59 ± 0.15	0.68 ± 0.15	0.62 ± 0.23
Ultimate load (N)	0.33 ± 0.14	0.40 ± 0.21	0.51 ± 0.24
Energy absorbed (J)	0.19 ± 0.10	0.35 ± 0.18	0.44 ± 0.24
Ultimate elongation (mm)	0.56 ± 0.11	0.68 ± 0.28	0.88 ± 0.39

MCL = medial collateral ligament; PDGF-BB = platelet-derived growth factor isomer BB.

optimal dosing of the two molecules or that multiple doses over the healing period were required. The latter of these is perhaps most important as the three growth factors have somewhat different temporal profiles. PDGF exerts the greatest of its effects almost immediately after injury occurs, triggering the healing cascades during inflammation that mark the beginning of healing proper, whereas IGF-I and bFGF are important during the intermediate and later phases, particularly during cell proliferation and angiogenesis. An optimum therapy using these molecules would most likely involve the immediate addition of PDGF.followed sometime later by the application of bFGF and/or IGF-I to up-regulate these later stages.

Subsequent to this study, Hildebrand et al.[47] demonstrated that the introduction of PDGF-BB into the injury site of the MCL of rabbits significantly increases its quality of healing, as shown by increases in the ultimate load, energy absorbed to failure, and ultimate elongation values of the femur-MCL-tibia complex (table III). However, these improved biomechanical properties were not apparent from histological examination as there was no significant difference in cellularity, vascularity or fibre alignment between treated ligaments and controls. It was thought that other structural components not examined, such as fibril diameters, must have been responsible for the increases.

This study was of interest as it also tested for a dose-dependent response using a fibrin sealant as a delivery vehicle to provide either 0, 0.4, or 20μg of PDGF-BB to the wound site. The high-dose treatment did indeed result in a femur-MCL-tibia complex with better biomechanical properties than the low-dose, successfully demonstrating a positive dose-response.

2.4 bFGF

Chan et al.[24] studied the effects of a single injection of bFGF on type III collagen expression, cell proliferation, ultimate stress and the pyridinoline content in the initial stages of healing in the rat patellar tendon. Three days after a defect was introduced into the mid-part of the petallar tendon, various doses of bFGF were injected directly into the wound site. It was observed that after 7 days increasing dosage of bFGF was correlated with increases in collagen type III expression and cellular proliferation, and although ultimate stress and pyridinoline content appeared to also increase, it was not found to be statistically significant.

In a study by Fukui et al.,[54] a defect in the MCL was treated with varying doses of recombinant human bFGF carried by a fibrin gel, and repair tissues examined at postoperative days 7, 14, 21 and 42. bFGF was found to promote the early formation of repair tissue compared with controls. Again, a dose-dependent response was shown, although in

this case higher doses had adverse effects. While a low dose resulted in the rate of tissue maturation being very similar to the controls, a high dose resulted in significant delays in maturation observed at the third and sixth week. A further observation was that type I procollagen expression was reduced in all bFGF-treated groups.

Somewhat similar results were found by Kobayashi et al.[55] in an investigation of the healing canine anterior cruciate ligament. In this work, cylindrical defects were introduced into the anteromedial bundle of the canine anterior cruciate ligament, a region known to have an extremely poor potential for healing, and treated with bFGF-impregnated pellets. The early stages of healing were shown to be positively influenced by the treatment, with defects quickly filling with new granulation tissue, as opposed to only partial filling in the control group. A dose-response was not investigated in this study, and although the amount of bFGF used was identical to the highest dose used in Fukui et al.'s[54] work and the progression of healing observed at the same time points, no significant disruption of maturation was reported as was in Fukui et al.'s study. These divergent results are likely due to the different animal models and/or delivery vehicle used in the two studies (for example Kobayashi et al.[55] postulated that it was unlikely that the bFGF pellet remained biologically active for more than 3 weeks, and Fukui et al.[54] only observed the interference of repair tissue maturation from week 3 onwards), as well as the different environments of the two ligaments studied (compared with the MCL, the anterior cruciate ligament has a poor supply of blood and early repair cells due to its intra-articular location[56]).

Kobayashi et al.[55] also noted that bFGF provided only a boost to the initial stages of healing, yet all subsequent steps proceeded with significantly more speed and efficacy than would take place naturally. It was hypothesised therefore that this initial 'kick-start' was all that was required to set in motion a cascade of other stimuli, most probably derived from invading cells and surrounding fluid, which resulted in greatly improved healing. This suggests that potential therapies may only have to control a small group or even a single key molecule to instigate or accelerate healing at a recalcitrant wound site – an important attribute for effective, practical, and economical therapies.

3. Future Directions

Each of the five growth factors discussed in sections 1 and 2 has important, varied roles within the healing tendon. IGF-I, PDGF and bFGF have vital functions during the early and intermediate stages of healing, during which they aid in the migration and proliferation of fibroblasts and stimulate extracellular matrix synthesis. TGFβ and VEGF also have some role in these processes, and in addition are instrumental in the remodelling phases, regulating angiogenesis within the wound site. Each of these molecules is involved in a myriad of interactions during these different stages of tendon healing, affecting both its own activity and expression as well as that of other molecules. If growth factors are to be successfully employed as therapeutics in the future, further research will be required. Work will need to focus on further defining the roles of each of the growth factors known as well as the strategies of regulation they employ, and most importantly will need to identify and clarify the synergistic and antagonistic influences they have on one another.

Some success has already been achieved utilising growth factors as therapeutics using a variety of delivery techniques, including direct injection, surgical implant, collagen or gel vehicles and gene therapy. In most of these studies, the application of a single molecule has shown some enhancement of healing; however, in general this temporary boost of a single 'healing signal' soon becomes diluted out, and has only a limited effect on the final outcome. Using a combination of patients' own growth factors to promote healing in injured tissue has become an important and potentially very fruitful area of research. Autologous growth factors are produced by platelets which are easily harvested from whole blood by a few centrifugation steps. Once a platelet-rich plasma specimen has been prepared, platelets can be activated to produce high titre growth factor

combinations which can then be delivered to the wound site. While little work has been performed on this type of growth factor treatment specifically for tendon and ligament healing, several studies on other tissues have shown promise. Tischler[57] for example, used autologous PDGF to treat decubitus ulcers, and observed much higher rates of healing in treated ulcers verses controls. Obviously the treatment in this study simply involved the topical application of the growth factor combination onto the wound site – delivering it to a healing ligament or tendon would present more of a problem.

In future, most success will likely come from the application of not one but multiple growth factors over the healing period in a similar way. A treatment programme could be envisioned in which a key molecule could be applied at the beginning of each phase of healing to significantly compress the healing process while simultaneously increasing the quality. Inherent in this approach however, is the problem of a subject requiring multiple treatments over a relatively short time. In this case, treatments involving surgery to deliver the molecule would most likely be infeasible; a direct injection of either the molecule or gene as part of a gene therapy solution would be more satisfactory. These options of course present their own problems, the most obvious of which is the need for sustained yet controlled release (or production) of the therapeutic molecule.

A viable alternative to the exogenous application of growth factors would of course be to use some other kind of stimuli to increase the production or activity of endogenous growth factors. Studies in this area have already been undertaken in a number of wound healing models using various stimuli, including hypoxia, ultrasound, mechanical, and electrical stimulation. Recently, Bouletreau et al.[58] used hypoxia, a stimuli present in the microenvironment of a fracture, to increase the production of bone morphogenetic protein-2 transcripts in cultured bovine endothelial cells. A 2- to 3-fold increase in bone morphogenetic protein-2 mRNA expression was observed after 24 and 48 hours in hypoxic cells compared with controls. Likewise, a study by Yeung et al.[59] showed TGFβ-1 production could be increased in response to mechanical stimulation in distracted fracture callus cells compared with normal fracture callus cells. These and other studies have shown that different types of physical and chemical stimuli are effectively translated into biological stimuli that result in the activation of the normal growth factor-mediated healing cascades. The use of these techniques to activate or amplify endogenous growth factors coupled with an effective exogenous application could prove to be extremely beneficial and obviate the need for invasive surgical procedures.

4. Conclusion

The processes of tendon and ligament healing are highly complex, but are slowly starting to become more well defined. Of the large number of molecules involved, growth factors play a central role. They are a diverse group of signal molecules whose effects are intricate and overlapping, and whose action is often dependent on dose, temporal expression, interaction with other growth factors, and even spatial distribution at the injury site.

There has been a steadily increasing number of *in vitro* and *in vivo* investigations into the action of growth factors over recent years which have provided vital information on the mechanics of the healing process. These data have been used to perform *in vivo* growth factor-based therapeutic studies which have shown some definite promise in increasing the efficiency and effectiveness of tendon healing. While a truly practical and effective treatment based on the application or regulation of growth factors *in vivo* may still be some time away, the future of cytokine-based therapies is promising.

Acknowledgements

The authors would like to acknowledge support provided by St George Hospital and South Eastern Area Health Service.

References

1. Albright JA. The scientific basis of orthopaedics. 2nd ed. Norwalk (CT): Appleton & Lange, 1987

2. Mast BA. Healing in other tissues. Surg Clin North Am 1997; 77 (3): 529-47
3. Hyman J, Rodeo SA. Injury and repair of tendons and ligaments. Phys Med Rehabil Clin N Am 2000; 11 (2): 267-88
4. Woo SL, Hildebrand K, Watanabe N, et al. Tissue engineering of ligament and tendon healing. Clin Orthop 1999; 367S: S312-23
5. Klein MB, Pham H, Yalamanchi N, et al. Flexor tendon wound healing in vitro: the effect of lactate on tendon cell proliferation and collagen production. J Hand Surg [Am] 2001; 26A: 847-54
6. Braddock M, Campbell C, Zuder D. Current therapies for wound healing: electrical stimulation, biological therapeutics and the potential for gene therapy. Int J Dermatol 1999; 38: 808-17
7. Braddock M. The transcription factor Egr-1: a potential drug in wound healing and tissue repair. Ann Med 2001; 33 (5): 313-8
8. Sciore P, Boykiw R, Hart DA. Semi-quantitive reverse transcriptase polymerase chain reaction analysis of mRNA for growth factors and growth factor receptors from normal and healing rabbit medial collateral ligament tissue. J Orthop Res 1998; 16: 429-37
9. Hansson HA, Dahlin L, Lundborg G, et al. Transiently increased insulin-like growth factor I immunoreactivity in tendons after vibration trauma: an immunohistochemical study on rats. Scand J Plast Reconstr Surg Hand Surg 1988; 22 (1): 1-6
10. Lynch SE, Colvin R, Antoniades HN. Growth factors in wound healing: single and synergistic effects on partial thickness porcine skin wounds. J Clin Invest 1989; 84 (2): 640-6
11. Jones JI, Clemmons D. Insulin-like growth factors and their binding proteins: biological actions. Endocr Rev 1995; 16 (1): 3-34
12. Abrahamsson SO. Similar effects of recombinant human insulin-like growth factor-I and II on cellular activities in flexor tendons of young rabbits: experimental studies in vitro. J Orthop Res 1997; 15 (2): 256-62
13. McCarthy TL, Centrella M, Canalis E. Regulatory effects of insulin-like growth factors I and II on bone collagen synthesis in rat calvarial cultures. Endocrinology 1989; 124 (1): 301-9
14. Chang J, Thunder R, Most D, et al. Studies in flexor tendon wound healing: neutralizing antibody to TGF-B1 increases postoperative range of motion. Plast Reconstr Surg 2000; 105 (1): 148-55
15. Bennett NT, Schultz G. Growth factors and wound healing: biochemical properties of growth factors and their receptors. Am J Surg 1993; 165 (6): 728-37
16. Wojciak B, Crossan J. The effects of T cells and their products on in vitro healing of epitenon cell microwounds. Immunology 1994; 83 (1): 93-8
17. Zhu X, Hu C, Zhang Y, et al. Expression of cyclin-dependent kinase inhibitors p21 (cip1) and p27 (kip1), during wound healing in rats. Wound Repair Regen 2001; 9: 205-12
18. Marui T, Niyibizi C, Georgescu HI, et al. Effect of growth factors on matrix synthesis by ligament fibroblasts. J Orthop Res 1997; 15 (1): 18-23
19. Natsu-ume T, Nakamura N, Shino K, et al. Temporal and spatial expression of transforming growth factor-beta in the healing patellar ligament of the rat. J Orthop Res 1997; 15 (6): 837-43
20. Boyer MI, Watson J, Lou J, et al. Quantitative variation in vascular endothelial growth factor mRNA expression during early flexor tendon healing: an investigation in a canine model. J Orthop Res 2001; 19 (5): 869-72
21. Gelberman RH, Khabie V, Cahill CJ. The revascularization of healing flexor tendons in the digital sheath: a vascular injection study in dogs. J Bone Joint Surg Am 1991; 73 (6): 868-81
22. Pierce GF, Mustoe T, Lingelbach J, et al. Platelet-derived growth factor and transforming growth factor-beta enhance tissue repair activities by unique mechanisms. J Cell Biol 1989; 109 (1): 429-40
23. Folkman J, Klagsbrun M. Angiogenic factors. Science 1987; 235 (4787): 442-7
24. Chan BP, Fu S, Qin L, et al. Effects of basic fibroblast growth factor (bFGF) on early stages of tendon healing: a rat patellar tendon model. Acta Orthop Scand 2000; 71 (5): 513-8
25. Chang J, Most D, Thunder R, et al. Molecular studies in flexor tendon wound healing: the role of basic fibroblast growth factor gene expression. J Hand Surg [Am] 1998; 23A (6): 1052-9
26. Winston BW, Krein P, Mowat C, et al. Cytokine-induced macrophage differentiation: a tale of 2 genes. Clin Invest Med 1999; 22 (6): 236-55
27. Le Roth D. Insulin-like growth factors. N Engl J Med 1997; 336 (9): 633-9
28. Cohick WS, Clemmons DR. The insulin-like growth factors. Annu Rev Physiol 1993; 55: 131-53
29. Steenfos H, Hunt T. Insulin-like growth factor has a major role in wound healing. Surg Forum 1989; 40: 68-70
30. Tsuzaki M, Brigman B, Yamamoto J, et al. Insulin-like growth factor-I is expressed by avian flexor tendon cells. J Orthop Res 2000; 18 (4): 546-56
31. Edwall D, Schalling M, Jennische E, et al. Induction of insulin-like growth factor I messenger ribonucleic acid during regeneration of rat skeletal muscle. Endocrinology 1989; 124: 820-5
32. Fortier LA, Balkman C, Sandell LJ, et al. Insulin-like growth factor-I gene expression patterns during spontaneous repair of acute articular cartilage injury. J Orthop Res 2001; 19 (4): 720-8
33. Bos PK, van Osch G, Frenz DA, et al. Growth factor expression in cartilage wound healing: temporal and spatial immunolocalization in a rabbit auricular cartilage wound model. Osteoarthritis Cartilage 2001; 9 (4): 382-9
34. Vogt PM, Lehnhardt M, Wagner D, et al. Growth factors and insulin-like growth factor binding proteins in acute wound fluid. Growth Horm IGF Res 1998; 8 Suppl B: 107-9
35. Rubini M, Werner H, Gandini E, et al. Platelet-derived growth factor increases the activity of the promoter of the insulin-like growth factor-I (IGF1) receptor gene. Exp Cell Res 1994; 211: 374-9
36. Bottinger EP, Letterio J, Roberts AB. Biology of TGF-Beta in knockout and transgenic mouse models. Kidney Int 1997; 51: 1355-60
37. Ngo M, Pham H, Longaker MT, et al. Differential expression of transforming growth factor-beta receptors in a rabbit zone II flexor tendon wound healing model. Plast Reconstr Surg 2001; 108: 1260-7
38. Klein MB. Flexor tendon healing in vitro: effects of TGF-(beta) on tendon cell collagen production. J Hand Surg [Am] 2002; 27A (4): 615-21
39. Centrella M, McCarthy T, Canalis E. Transforming growth factor-beta and remodeling of bone. J Bone Joint Surg Am 1991; 73A: 1418-28
40. Jackson JR, Minton J, Ho ML, et al. Expression of vascular endothelial growth factor in synovial fibroblasts is induced by hypoxia and interleukin 1beta. J Rheumatol 1997; 24 (7): 1253-9

41. Ellis LM, Takahashi Y, Liu W, et al. Vascular endothelial growth factor in human colon cancer: biology and therapeutic implications. Oncologist 2000; 5 Suppl. 1: 11-5

42. Clauss M, Weich H, Breier G. The vascular endothelial growth factor receptor Flt-1 mediates biological activities: implications for a functional role of placenta growth factor in monocyte activation and chemotaxis. J Biol Chem 1996; 271: 17629-34

43. Deroanne CF, Hajitou A, Calberg-Bacq CM, et al. Angiogenesis by fibroblast growth factor 4 is mediated through an autocrine up-regulation of vascular endothelial growth factor expression. Cancer Res 1997; 57 (24): 5590-7

44. Duffy Jr FJ, Seiler J, Gelberman RH, et al. Growth factors and canine flexor tendon healing: initial studies in uninjured and repair models. J Hand Surg [Am] 1995; 20 (4): 645-9

45. Ronnstrand L, Heldin C. Mechanisms of platelet-derived growth factor-induced chemotaxis. Int J Cancer 2001; 91 (6): 757-62

46. Yoshikawa Y, Abrahamsson S. Dose-related cellular effects of platelet-derived growth factor-BB differ in various types of rabbit tendons in vitro. Acta Orthop Scand 2001; 72 (3): 287-92

47. Hildebrand KA, Woo SL, Smith DW, et al. The effects of platelet-derived growth factor-BB on healing of the rabbit medial collateral ligament: an in vivo study. Am J Sports Med 1998; 26 (4): 549-54

48. Nugent MA, Iozzo R. Fibroblast growth factor-2. Int J Biochem Cell Biol 2000; 23: 115-20

49. Chan BP, Chan K, Maffulli N, et al. Effect of basic fibroblast growth factor: an in vitro study of tendon healing. Clin Orthop 1997; 342: 239-47

50. Kurtz CA, Loebig T, Anderson DD, et al. Insulin-like growth factor I accelerates functional recovery from Achilles tendon injury in a rat model. Am J Sports Med 1999; 27 (3): 363-9

51. Letson AK, Dahners L. The effect of combinations of growth factors on ligament healing. Clin Orthop 1994; 308: 207-12

52. Forslund C, Aspenberg P. Tendon healing stimulated by injected CDMP-2. Med Sci Sports Exerc 2001; 33 (5): 685-7

53. Aspenberg P, Forslund C. Bone morphogenetic proteins and tendon repair. Scand J Med Sci Sports 2000; 10 (6): 372-5

54. Fukui N, Katsuragawa Y, Sakai H, et al. Effect of local application of basic fibroblast growth factor on ligament healing in rabbits. Rev Rhum Engl Ed 1998; 65 (6): 406-14

55. Kobayashi D, Kurosaka M, Yoshiya S, et al. Effect of basic fibroblast growth factor on the healing of defects in the canine anterior cruciate ligament. Knee Surg Sports Traumatol Arthrosc 1997; 5 (3): 189-94

56. Hefti FL, Kress A, Fasel J, et al. Healing of the transacted anterior cruciate ligament in the rabbit. J Bone Joint Surg Am 1991; 73: 373-83

57. Tischler M. Platelet rich plasma: the use of autologous growth factors to enhance bone and soft tissue grafts. N Y State Dent J 2002 Mar; 68 (3): 22-4

58. Boulletreau PJ, Warren SM, Spector JA, et al. Hypoxia and VEGF up-regulate BMP-2 mRNA and protein expression in microvascular endothelial cells: implications for fracture healing. Plast Reconstr Surg 2002 Jun; 109 (7): 2384-97

59. Yeung HY, Lee KM, Fung KP, et al. Sustained expression of transforming growth factor-beta1 by distraction during distraction osteogenesis. Life Sci 2002 May 24; 71 (1): 67-79

Correspondence and offprints: Prof. *George A.C. Murrell*, Department of Orthopaedic Surgery, St George Hospital, Kogarah, Sydney, 2217, Australia.
E-mail: admin@ori.org.au

CORRESPONDENCE

Tumor Necrosis Factor–α Increased Production during Thalidomide Treatment in Patients with Tuberculosis and Human Immunodeficiency Virus Coinfection

To the Editor—We read with interest the article by Bekker et al. [1] on the role of thalidomide-induced antigen-specific immune stimulation in patients with human immunodeficiency virus (HIV) and *Mycobacterium tuberculosis* coinfection. In their report, it is suggested that thalidomide treatment of HIV-infected patients does not reduce plasma tumor necrosis factor (TNF)–α levels. The observation is explained by a differential effect of thalidomide on monocyte and T cell TNF-α production. In particular, the authors report that thalidomide inhibited TNF-α production by lipopolysaccharide-stimulated monocytes but failed to inhibit TNF-α production by activated T cells [2–5]. Finally, the authors found an increase in TNF-α production at day 21 of therapy in the thalidomide group, thus suggesting that the drug could be responsible for this increase by stimulation of T cell activation.

As Bekker et al. observed, these data seem to be in contrast with the findings of previous studies, mainly performed in vitro, which reported an anti-inflammatory effect of thalidomide, mediated by an inhibition of TNF-α production [4, 6]. Nevertheless, the data confirm the most recent in vivo reports showing an increase in TNF-α concentrations and soluble TNF-α receptors during thalidomide treatment [7, 8]. These data suggest that thalidomide is not a systemic TNF-α inhibitor. Moreover, it must be underlined that increased TNF-α production in the thalidomide-treated patients was associated with unexplained deaths when thalidomide was used in the treatment of toxic epidermal necrolysis [7].

We conducted a study, similar to the one performed by Bekker and colleagues, using thalidomide to treat HIV- and tuberculosis-coinfected patients. We studied 6 HIV- and *M. tuberculosis*–infected patients, characterized by a poor response to the antituberculosis treatment, who were subsequently treated with thalidomide as adjuvant therapy. Immunological evaluations prior to thalidomide introduction suggested that these patients had significantly lower levels of TNF-α production than did the control subjects. Following thalidomide treatment, we observed a progressive increase in TNF-α production with a peak after about day 35 of therapy, thus confirming the observations made by Bekker and colleagues [1]. The increase in TNF-α concurred with a significant recovery of Th1 cytokine production, such as interleukin-2 and interferon-γ, and was followed by a significant improvement in clinical conditions (reduction of fever, increase in body weight, improvement in radiological findings, and negativization of *M. tuberculosis* cultures) in all patients.

Our data, along with the results of Bekker et al., emphasize the usefulness of thalidomide treatment in patients infected by *M. tuberculosis* and demonstrate the complexity of the immunomodulating effects mediated by this drug. In agreement with the more recent in vivo reports, we confirm that thalidomide did not reduce TNF-α levels, and this is probably due to an activation of T cell activity, as hypothesized by Bekker and colleagues. However, these data raise questions as to the nature of the interaction between TNF-α and thalidomide. In the light of these results, we suggest extreme caution in undertaking studies that support the clinical use of thalidomide, on the basis of the assumption of its contradictory role in TNF-α inhibition.

Andrea Gori,[1] Maria Cristina Rossi,[1] Daria Trabattoni,[2] Giulia Marchetti,[1] Maria Luisa Fusi,[2] Chiara Molteni,[1] Mario Clerici,[2] and Fabio Franzetti[1]

[1]Institute of Infectious Diseases and Tropical Medicine and [2]Chair of Immunology, "Luigi Sacco" Hospital, University of Milan, Milan, Italy

References

1. Bekker L-G, Haslett P, Maartens G, Steyn L, Kaplan G. Thalidomide-induced antigen-specific immune stimulation in patients with human immunodeficiency virus type 1 and tuberculosis. J Infect Dis 2000;181:954–65.
2. Sampaio EP, Kaplan G, Miranda A, et al. The influence of thalidomide on the clinical and immunological manifestation of erythema nodosum leprosum. J Infect Dis 1993;168:408–14.
3. Tramontana JM, Utaipat U, Molloy A, et al. Thalidomide treatment reduces tumor necrosis factor α production and enhances weight gain in patients with pulmonary tuberculosis. Mol Med 1995;1:384–97.
4. Sampaio EP, Sarno EN, Galilly R, et al. Thalidomide selectively inhibits tumor necrosis factor α production by stimulated human monocytes. J Exp Med 1991;173:699–703.
5. Haslett PAJ, Corral LG, Albert M, et al. Thalidomide costimulates primary human T lymphocytes, preferentially inducing proliferation, cytokine production, and cytotoxic responses in the $CD8^+$ subset. J Exp Med 1998; 187:1885–92.
6. Klausner JD, Freedman VH, Kaplan G. Thalidomide as an anti–TNF-alpha inhibitor: implications for clinical use. Clin Immunol Immunopathol 1996; 81:219–23.
7. Wolkenstein P, Latarjet J, Roujeau JC, et al. Randomised comparison of thalidomide versus placebo in toxic epidermal necrolysis. Lancet 1998;352: 1586–9.
8. Jacobson JM, Spritzler J, Fox L, et al. Thalidomide for the treatment of esophageal aphthous ulcers in patients with human immunodeficiency virus infection. J Infect Dis 1999;180:61–7.

Financial support: This work was supported in part by a grant from the Italian National Institute of Health, "National Research Program on AIDS."

Reprints or correspondence: Dr. Andrea Gori, Institute of Infectious Diseases and Tropical Medicine, University of Milan, "L. Sacco" Hospital, Via G.B. Grassi 74 20157, Milan, Italy (andrea.gori@unimi.it).

The Journal of Infectious Diseases 2000;182:639

0022-1899/2000/18202-0039$02.00

Reply

To the Editor—We thank Gori et al. [1] for their letter concerning our recently published article [2]. We have read the letter with great interest. Although we do not have any specific response to the letter, we would like to suggest 3 recently published references relevant to their commentary [3–5].

Gilla Kaplan
Laboratory of Cellular Physiology and Immunology, The Rockefeller University, New York, New York

References

1. Gori A, Rossi MC, Trabattoni D, et al. Tumor necrosis factor–α increased production during thalidomide treatment in patients with tuberculosis and human immunodeficiency virus coinfection. J Infect Dis 2000;182:639 (in this issue).
2. Bekker L-G, Haslett P, Maartens G, Steyn L, Kaplan G. Thalidomide-induced antigen-specific immune stimulation in patients with human immunodeficiency virus type 1 and tuberculosis. J Infect Dis 2000;181:954–65.
3. Klausner JD, Kaplan G, Haslett PAJ. Thalidomide in toxic epidermal necrosis (letter). Lancet 1999;353:324.
4. Haslett PAJ, Klausner JD, Makonkawkeyoon S, et al. Thalidomide stimulates T cell responses and interleukin-12 production in HIV-infected patients. AIDS Res Hum Retroviruses 1999;15:1169–79.
5. Haslett PAJ, Nixon DF, Shen Z, et al. Strong human immunodeficiency virus (HIV)–specific $CD4^+$ T cell responses in a cohort of chronically infected patients are associated with interruptions in anti-HIV chemotherapy. J Infect Dis 2000;181:1264–73.

Reprints or correspondence: Dr. Gilla Kaplan, Laboratory of Cellular Physiology and Immunology, The Rockefeller University, 1230 York Ave., New York, NY 10021 (kaplang@rockvax.rockefeller.edu).

The Journal of Infectious Diseases 2000;182:640

0022-1899/2000/18202-0040$02.00

Diagnostic and Therapeutic Injection of the Hip and Knee

DENNIS A. CARDONE, D.O., C.A.Q.S.M., and ALFRED F. TALLIA, M.D., M.P.H.
University of Medicine and Dentistry of New Jersey, New Brunswick, New Jersey

Joint injection of the hip and knee regions is a useful diagnostic and therapeutic tool for the family physician. In this article, the injection procedure for the greater trochanteric bursa, the knee joint, the pes anserine bursa, the iliotibial band, and the prepatellar bursa is reviewed. Indications for greater trochanteric bursa injection include acute and chronic inflammation associated with osteoarthritis, rheumatoid arthritis, repetitive use, and other traumatic injuries to the area. For the knee joint, aspiration may be performed to aid in the diagnosis of an unexplained effusion and relieve discomfort caused by an effusion. Injection of the knee can be performed for viscosupplementation or corticosteroid therapy. Indications for corticosteroid injection include advanced osteoarthritis and other inflammatory arthritides, such as gout or calcium pyrophosphate deposition disease. Swelling and tenderness of pes anserine or prepatellar bursae can be relieved with aspiration and corticosteroid injection. Persistent pain and disability from iliotibial band syndrome respond to local injection therapy. The proper technique, choice and quantity of pharmaceuticals, and appropriate follow-up are essential for effective outcomes. (Am Fam Physician 2003;67:2147-52. Copyright© 2003 American Academy of Family Physicians.)

This article is one in a series of "Office Procedures" articles coordinated by Dennis A. Cardone, D.O., C.A.Q.S.M., associate professor, and Alfred F. Tallia, M.D., M.P.H., associate professor, Department of Family Medicine, UMDNJ–Robert Wood Johnson Medical School, New Brunswick, New Jersey.

This article, part of a series on diagnostic and therapeutic injections, reviews the hip and knee regions. The rationale, indications, contraindications, and general approach to this technique are discussed in the first article of the series.[1] The hip and knee are sites of multiple injuries and inflammatory conditions[2] that lend themselves to diagnostic and therapeutic injection.[3-8] Intra-articular injection of the hip is rarely performed by family physicians because this procedure is commonly performed with fluoroscopic guidance. This article focuses on the anatomy, pathology, diagnosis, and injection technique of the common sites for which this skill is applicable, including the greater trochanteric bursa, knee joint, pes anserine bursa, iliotibial band, and the prepatellar bursa.

Greater Trochanteric Bursa

ANATOMY

The trochanteric bursa is located over the lateral prominence of the greater trochanter of the femur.

INDICATIONS

Trochanteric bursitis, the primary indication for therapeutic injection at this site, usually is associated with chronic pressure or trauma to the area. Leg-length abnormalities, obesity, rheumatoid arthritis, and osteoarthritis are associated factors in many patients.[9] Friction from a tight iliotibial band, typically seen in runners, also can cause this problem. Diagnosis is confirmed by palpation of tenderness, and sometimes swelling, in the region of the bursa.

TIMING AND OTHER CONSIDERATIONS

Early corticosteroid injection frequently is the preferred treatment, because it has been shown to be effective with satisfactory duration of effect.[10]

TECHNIQUE

See *Table 1* for a list of pharmaceuticals and equipment.

Position of Patient. The patient should be in the lateral recumbent position with the affected side up. For the patient's comfort and

TABLE 1
Equipment and Pharmaceuticals

Site	*Syringe*	*Needle*	*Anesthetic*	*Corticosteroid**	*Hydrocortisone equivalents per injection*
Greater trochanteric bursa	5 to 10 mL	22 or 25 gauge, 1.5 inch (longer if patient is very obese)	3 to 5 mL of 1 percent lidocaine (Xylocaine) or 0.25 or 0.5 percent bupivacaine (Marcaine)	1 mL betamethasone sodium phosphate and acetate (Celestone Soluspan) *or*	150
				1 mL methylprednisolone (Depo-Medrol), 40 mg per mL	200
Knee joint	30 to 60 mL (for aspiration), 10 mL (for injection)	18, 20, or 22 gauge 1.5 inch†	5 to 7 mL of 1 percent lidocaine or 0.25 or 0.5 percent bupivacaine	2 to 3 mL betamethasone sodium phosphate and acetate *or*	300 to 450
				2 to 3 mL methylprednisolone, 40 mg per mL	400 to 600
Pes anserine bursa	5 mL	25 gauge, 1 inch	2 mL of 1 percent lidocaine or 0.25 or 0.5 percent bupivacaine	1 mL betamethasone sodium phosphate and acetate *or*	150
				1 mL methylprednisolone, 40 mg per mL	200
Iliotibial band	5 mL	25 gauge, 1 inch	1 to 2 mL of 1 percent lidocaine or 0.25 or 0.5 percent bupivacaine	1 mL betamethasone sodium phosphate and acetate *or*	150
				1 mL methylprednisolone, 40 mg per mL	200
Prepatellar bursa	20 to 30 mL (for aspiration), 5 to 10 mL (for injection)	18 or 22 gauge, 1 or 1.5 inch†	2 mL of 1 percent lidocaine or 0.25 or 0.5 percent bupivacaine	1 mL betamethasone sodium phosphate and acetate *or*	150
				1 mL methylprednisolone, 40 mg per mL	200

*—*Other preparations such as triamcinolone (Aristospan) or dexamethasone may be used.*
†—*For aspiration of large effusions, a hemostat is needed to immobilize the needle when removing the syringe to empty it. A hemostat also is used to stabilize the needle when changing the syringe to inject after aspiration. The larger bore needle is used for aspiration.*

The Authors

DENNIS A. CARDONE, D.O., C.A.Q.S.M., is associate professor and director of sports medicine and the sports medicine fellowship in the department of family medicine at the University of Medicine and Dentistry of New Jersey (UMDNJ)–Robert Wood Johnson Medical School, New Brunswick. Dr. Cardone is a graduate of the New York College of Osteopathic Medicine, Old Westbury, N.Y., and completed his residency at the UMDNJ–Robert Wood Johnson Medical School Family Medicine Residency. He completed his sports medicine fellowship at UMDNJ.

ALFRED F. TALLIA, M.D., M.P.H., is associate professor and vice chair in the department of family medicine at the UMDNJ–Robert Wood Johnson Medical School, New Brunswick, N.J. Dr. Tallia is a graduate of the UMDNJ–Robert Wood Johnson Medical School and completed his residency at the Thomas Jefferson University Family Medicine Residency, Philadelphia. He received his public health degree at Rutgers University, New Brunswick, N.J.

Address correspondence to Dennis A. Cardone, D.O., C.A.Q.S.M., Dept. of Family Medicine, UMDNJ, 1 Robert Wood Johnson Place, MEB288, New Brunswick, NJ 08903 (e-mail: cardonda@umdnj.edu). Reprints are not available from the authors.

stabilization, the hip is flexed 30 to 50 degrees and the knee is flexed 60 to 90 degrees.

Palpation of Landmarks. The greater trochanter is identified by palpating the femur from the mid-shaft proximally until the area of bony protrusion is reached. The injection site is the point of maximal tenderness or swelling.

Approach and Needle Entry. At the area most tender or swollen to palpation in the region of the greater trochanter, a 22- or 25-gauge, one and one-half–inch needle is inserted perpendicular to the skin *(Figure 1)*. In very obese patients, a longer needle may be required. The needle should be inserted directly down to

FIGURE 1. Greater trochanteric bursa injection. The needle is inserted perpendicular to the skin and directed down to the point of maximal tenderness.

bone and then withdrawn two to three millimeters before injecting.

Knee Joint

ANATOMY

Two functional joints, the femoral-tibial and the femoral-patellar, make up the knee. Primary stabilizers of the knee are the anterior and posterior cruciate ligaments, the medial and lateral collateral ligaments, and the capsular ligaments.

INDICATIONS

Indications for aspiration include unexplained effusion, possible septic arthritis, and relief of discomfort caused by an effusion.[11] Indications for injection include corticosteroid delivery for advanced osteoarthritis and other noninfectious inflammatory arthritides such as gout or calcium pyrophosphate deposition disease, or the delivery of viscosupplementation therapy.[12-14] Viscosupplementation preparations such as hylan G-F 20 (Synvisc) come with prefilled syringes and are used to treat the pain of knee joint osteoarthritis. Viscosupplementation and corticosteroid therapies are not used concomitantly.

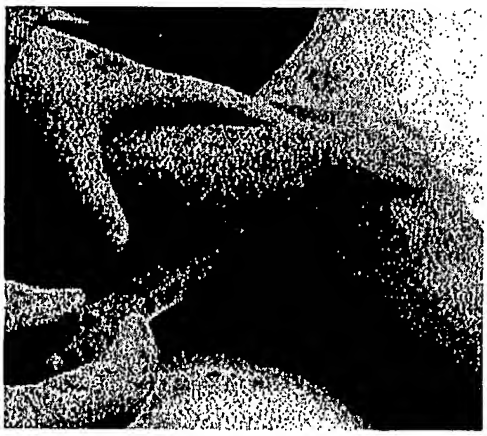

FIGURE 2. Lateral knee joint injection. Entry should be in the soft tissue between the patella and femur.

TIMING AND OTHER CONSIDERATIONS

The use of intra-articular corticosteroids is reserved for patients with more advanced disease and after other modalities have been tried. Aspiration for suspected septic arthritis must be performed immediately.

TECHNIQUE

See *Table 1* for a list of pharmaceuticals and equipment.

Position of Patient. The patient is in the supine position with the knee slightly flexed with a pillow or rolled towel in the popliteal space.

Palpation of Landmarks. Identify the medial, lateral, and superior borders of the patella.

Approach and Needle Entry. There are many different techniques for aspirating or injecting the knee. These include medial, lateral, and anterior approaches. Each has its own merit, but choice of approach is dependent on physician preference. The lateral approach is most commonly used and is illustrated here *(Figure 2)*. For this approach, lines are drawn along the lateral and proximal borders of the patella. The needle is inserted into the soft tissue between the patella and femur near the intersection point of the lines, and directed at a 45-degree angle toward the middle of the medial side of the joint. Before injection or aspiration, local anesthesia with lidocaine (Xylocaine) should be obtained. Always use sterile technique. Intra-articular injection flow should be even and without resistance.

For the medial approach, the needle enters the medial side of the knee under the middle

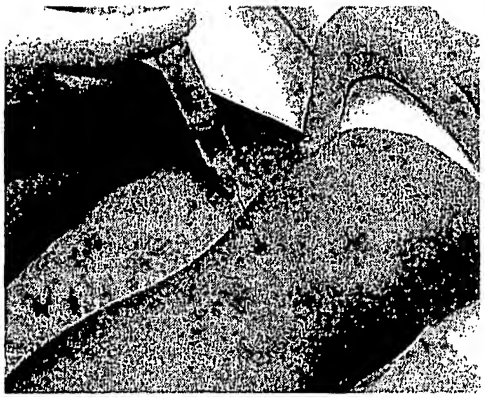

FIGURE 3. Per anserine bursa injection. The needle is inserted perpendicular to the tibia into the point of maximal tenderness.

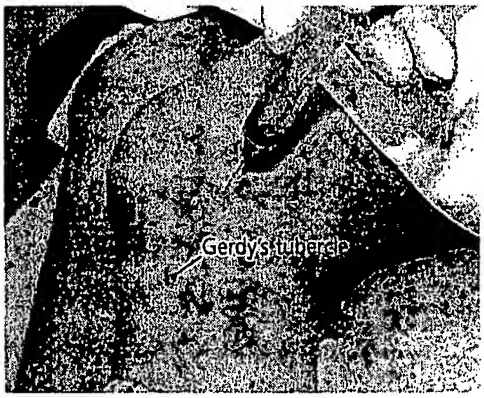

FIGURE 4. Iliotibial band injection. The needle is inserted at the point of maximal tenderness in the region of the lateral femoral condyle.

of the patella (midpole) and is directed toward the opposite patellar midpole. In the anterior approach, the knee is flexed 60 to 90 degrees, and the needle is inserted just medial or lateral to the patellar tendon and parallel to the tibial plateau. This technique is preferred by some physicians for its ease of joint entry in advanced osteoarthritis. However, the anterior approach may incur greater risk for meniscal injury by the needle.

Pes Anserine Bursa

ANATOMY

The pes anserine bursa is located between the medial collateral ligament and the sartorius, gracilis, and semitendinosus tendons. The bursa is located distal to the joint line in close proximity and posterior to the medial collateral ligament.

INDICATIONS

Direct trauma or repeated friction over the bursa can lead to inflammation. When the bursa is inflamed, contraction of the hamstring muscles, rotational movements of the tibia, and direct pressure over the pes anserine bursa usually will produce pain. The pain is in the posterior medial aspect of the knee distal to the joint space. This condition is more commonly seen in middle-aged or older overweight women, many with osteoarthritis of the knee.[15]

When injecting the knee joint using the anterior approach, the knee is flexed 60 to 90 degrees, and the needle is inserted just medial or lateral to the patellar tendon and parallel to the tibial plateau.

TIMING AND OTHER CONSIDERATIONS

Injection in this condition often is performed early in the course of treatment rather than after a trial of other modalities.

TECHNIQUE

See *Table 1* for a list of pharmaceuticals and equipment.

Position of Patient. The patient is in the supine position with the knee slightly flexed.

Palpation of Landmarks. Identify the tendinous border of the medial thigh muscles and follow them across the joint line to their insertion at the pes anserine.

Approach and Needle Entry. The needle is inserted perpendicular to the tibia into the point of maximal tenderness *(Figure 3)*. The needle is gently guided down to the bone and then withdrawn two to three millimeters to perform the injection.

Iliotibial Band

ANATOMY

The iliotibial band originates at the lateral iliac crest and inserts on the lateral proximal tibia. Gerdy's tubercle (sometimes called the lateral tubercle of the tibia) is a prominence of the anterior surface of the lateral condyle and is easily palpated just lateral to the distal portion of the patellar tendon.

INDICATIONS

Iliotibial band syndrome is a disorder of pain and degenerative changes that occurs as the iliotibial band repeatedly rubs over the prominence of the lateral femoral condyle. It is seen in activities with repetitive flexion and extension of the knee, such as running.[16-19]

Diagnosis is made by elicitation of tenderness over Gerdy's tubercle or the lateral femoral condyle. Ober's test is performed with the patient lying on the unaffected side. The affected hip is abducted against gravity, and the knee is flexed to 90 degrees with the knee and distal leg supported by the examiner's forearm and hand. The knee is then released. In a contracted iliotibial band, the knee does not drop with gravity.

TIMING AND OTHER CONSIDERATIONS

Injection is a later modality performed after a program of stretching of the iliotibial band, strengthening of the hip abductors, and modification of activity.[20,21]

TECHNIQUE

See *Table 1* for a list of pharmaceuticals and equipment.

Position of Patient. The patient is placed in the lateral recumbent position with the knee flexed 20 to 30 degrees.

Palpation of Landmarks. Along the lateral thigh, follow the course of the iliotibial band across the femoral condyle to its insertion at Gerdy's tubercle.

Approach and Needle Entry. The needle is inserted at the point of maximal tenderness in the region of the lateral condyle *(Figure 4)*.

Prepatellar Bursa

ANATOMY

The prepatellar bursa is located between the skin and the anterior surface of the patella, making it vulnerable to direct trauma.

INDICATIONS

Bursitis is manifested by swelling and tenderness anterior to the patella and can be the result of acute trauma.[22,23] Chronic prepatellar bursitis is more common than an acute episode and usually is the result of repeated episodes of microtrauma.[24] In the evaluation of a patient with prepatellar bursitis, the physician should be aware of possible underlying infection, fracture of the patella, or an associated intra-articular injury of the knee.[25,26] Bursitis-related prepatellar swelling must be differentiated from an intra-articular effusion. Aspiration of an inflamed bursa can be performed for relief of discomfort associated with a bursitis. If the symptoms of prepatellar bursitis are recurrent, corticosteroid injection may be performed.

TIMING AND OTHER CONSIDERATIONS

Aspiration of the prepatellar bursa may be performed acutely for relief of swelling and discomfort. Corticosteroid injection can be performed in cases of chronic or persistent bursitis after a trial of more conservative therapy.

TECHNIQUE

See *Table 1* for a list of pharmaceuticals and equipment.

Position of Patient. The patient is placed in the supine position with the knee resting in a comfortable position. A small pillow may be placed under the knee for comfort and support.

Palpation of Landmarks. The area over the patella is palpated for fluctuance.

Approach and Needle Entry. Aspiration and injection are performed by placing the needle directly into the fluid-filled bursa from the side *(Figure 5)*. Before injection with a corticosteroid, fluid should be aspirated from the

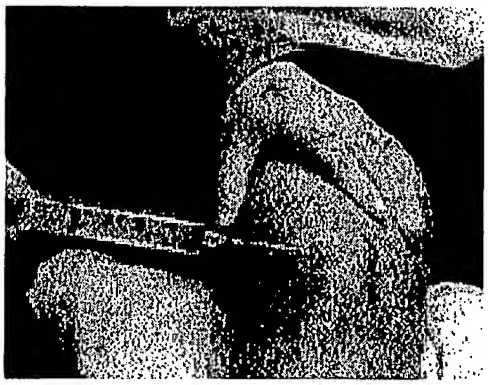

FIGURE 5. Prepatellar bursa injection. Entry is directly into the fluid-filled bursa, approached from the side.

If the symptoms of prepatellar bursitis are recurrent, corticosteroid injection may be performed.

bursa. If injecting, a hemostat is used to hold the needle in place while changing the syringe.

Follow-Up

Following aspiration of the prepatellar bursa, a pressure dressing should be applied, and the patient should remain in the supine position for several minutes. Following injection, the joint or injected region may be put through passive range of motion. The patient should remain in the office for 30 minutes after the injection to monitor for any adverse reactions. In general, patients should avoid strenuous activity involving the injected region for several days. Patients should be cautioned that they may experience worsening symptoms during the first 24 to 48 hours related to a possible steroid flare, which can be treated with ice and nonsteroidal anti-inflammatory drugs. Patients should be instructed against the application of heat. A follow-up appointment should be scheduled within three weeks.

The authors indicate that they do not have any conflicts of interest. Sources of funding: none reported.

REFERENCES

1. Cardone DA, Tallia AF. Joint and soft tissue injection. Am Fam Physician 2002;66:283-9,290.
2. Scopp JM, Moorman CT 3d. The assessment of athletic hip injury. Clin Sports Med 2001;20:647-59.
3. Adkins SB 3d, Figler RA. Hip pain in athletes. Am Fam Physician 2000;61:2109-18.
4. Kalb RL. Evaluation and treatment of hip pain. Hosp Pract 1998;33:131-2,135-6.
5. Agudelo CA, Wise CM. Crystal-associated arthritis in the elderly. Rheum Dis Clin North Am 2000; 26:527-46,vii.
6. Owen DS. Aspiration and injection of joints and soft tissues. In: Ruddy S, Harris ED Jr, Sledge CB, eds. Kelley's Textbook of rheumatology. 6th ed. Philadelphia: Saunders, 2001:583-603.
7. Genovese MC. Joint and soft-tissue injection. A useful adjuvant to systemic and local treatment. Postgrad Med 1998;103:125-34.
8. Zuckerman JD, Meislin RJ, Rothberg M. Injections for joint and soft tissue disorders: when and how to use them. Geriatrics 1990;45:45-52,55.
9. Shbeeb MI, Matteson EL. Trochanteric bursitis (greater trochanter pain syndrome). Mayo Clin Proc 1996;71:565-9.
10. Shbeeb MI, O'Duffy JD, Michet CJ Jr, O'Fallon WM, Matteson EL. Evaluation of glucocorticosteroid injection for the treatment of trochanteric bursitis. J Rheumatol 1996;23:2104-6.
11. Roberts WO. Knee aspiration and injection. The Physician and Sports Medicine 1998;26:93-4.
12. Vangsness CT Jr. Overview of treatment options for arthritis in the active patient. Clin Sports Med 1999;18:1-11.
13. Ravaud P, Moulinier L, Giraudeau B, Ayral X, Guerin C, Noel E, et al. Effects of joint lavage and steroid injection in patients with osteoarthritis of the knee: results of a multicenter, randomized, controlled trial. Arthritis Rheum 1999;42:475-82.
14. Wen DY. Intra-articular hyaluronic acid injections for knee osteoarthritis. Am Fam Physician 2000;62: 565-70,572.
15. Kang I, Han SW. Anserine bursitis in patients with osteoarthritis of the knee. South Med J 2000; 93:207-9.
16. Fredericson M, Cookingham CL, Chaudhari AM, Dowdell BC, Oestreicher N, Sahrmann SA. Hip abductor weakness in distance runners with iliotibial band syndrome. Clin J Sport Med 2000;10:169-75.
17. Almeida SA, Williams KM, Shaffer RA, Brodine SK. Epidemiological patterns of musculoskeletal injuries and physical training. Med Sci Sports Exerc 1999;31:1176-82.
18. Holmes JC, Pruitt AL, Whalen NJ. Iliotibial band syndrome in cyclists. Am J Sports Med 1993;21: 419-24.
19. Linenger JM, West LA. Epidemiology of soft-tissue/musculoskeletal injury among U.S. Marine recruits undergoing basic training. Mil Med 1992; 157:491-3.
20. Barber FA, Sutker AN. Iliotibial band syndrome. Sports Med 1992;14:144-8.
21. Sutker AN, Barber FA, Jackson DW, Pagliano JW. Iliotibial band syndrome in distance runners. Sports Med 1985;2:447-51.
22. Mysnyk MC, Wroble RR, Foster DT, Albright JP. Prepatellar bursitis in wrestlers. Am J Sports Med 1986;14:46-54.
23. Wroble RR, Mysnyk MC, Foster DT, Albright JP. Patterns of knee injuries in wrestling: a six year study. Am J Sports Med 1986;14:55-66.
24. Kerlan RK, Glousman RE. Injections and techniques in athletic medicine. Clin Sports Med 1989;8:541-60.
25. Pien FD, Ching D, Kim E. Septic bursitis: experience in a community practice. Orthopedics 1991;14: 981-4.
26. Wilson-MacDonald J. Management and outcome of infective prepatellar bursitis. Postgrad Med J 1987;63:851-3.

nature biotechnology

Small-scale systems for *in vivo* drug delivery

David A LaVan[1], Terry McGuire[2] & Robert Langer[3]

Recent developments in the application of micro- and nanosystems for drug administration include a diverse range of new materials and methods. New approaches include the on-demand activation of molecular interactions, novel diffusion-controlled delivery devices, nanostructured 'smart' surfaces and materials, and prospects for coupling drug delivery to sensors and implants. Micro- and nanotechnologies are enabling the design of novel methods such as radio-frequency addressing of individual molecules or the suppression of immune response to a release device. Current challenges include the need to balance the small scale of the devices with the quantities of drugs that are clinically necessary, the requirement for more stable sensor platforms, and the development of methods to evaluate these new materials and devices for safety and efficacy.

Drug delivery systems have already had an enormous impact on medical technology, greatly improving the performance of many existing drugs and enabling the use of entirely new therapies. Efforts to miniaturize drug delivery devices from the macroscale (>1 mm) to the microscale (100–0.1 μm) or nanoscale (100–1 nm) ultimately promise integrated systems that combine device technology with therapeutic molecules (small molecules, nucleic acids, peptides, proteins) to allow the creation of implantable devices that can monitor health status and provide prophylactic or therapeutic treatment *in situ*. At present, however, these efforts are constrained by several technological barriers.

New technologies and approaches are needed to manufacture devices that can deliver otherwise insoluble, unstable or unavailable therapeutic compounds, to reduce the amount of those compounds used, to localize the delivery of potent compounds, and to improve compliance by reducing the chances of missing or erring in a dose. From a logistical standpoint, devices must also not be so small that they are incapable of delivering the many drugs that require doses of microliters or more.

Currently, a typical dose of a potent drug is usually tens to hundreds of micrograms. For example, the adult EpiPen used to treat anaphylactic shock delivers an injection of 300 μg of epinephrine. Fentanyl, a powerful narcotic widely used for anesthesia and analgesia, is given in ~25 μg doses for a typical 50-kg adult. Drugs that are delivered in considerably larger quantities include common antibiotics, such as penicillin and amoxillin, which are typically given in doses exceeding 1 g/d for adults. Reducing scale quickly diminishes the volume available. A device that contains a reservoir that is a cube 1 mm on a side contains a volume of 1 μl, a cube 100 μm on a side holds 1 nl and a cube 10 μm on a side holds 1 pl.

To some degree, the limited capacity of small-scale delivery techniques may be remedied by using them in conjunction with larger reservoirs (in the microliter or milliliter range) or by relying on arrays of devices or compartments to provide sufficient volume. In addition, drugs, as currently prescribed, often contain carriers, flavoring agents, binders and coatings that may be unnecessary with more advanced delivery methods. Improvements in delivery efficiency and localization may also provide a solution to the capacity dilemma by reducing dosages.

The term 'small-scale' in the title of this article reflects the interdisciplinary and scale-bridging nature of the field—chemistry, microfabrication, biology and medicine all converge, each with their own sense of what is small. Classical divisions of macroscale, microscale and nanoscale are not necessarily helpful in describing and comparing drug delivery methods. Moreover, the literature does not provide a consistent distinction between microtechnology and nanotechnology. Some authors choose the size scale of 100 nm as the dividing line; others emphasize the nature of the synthesis—'top down' or 'bottom up'. Macro- and microscale fabrication is often considered a top-down process: the material is fabricated into its final shape from a larger piece through the removal of unwanted regions by machining or etching. Bottom-up synthesis, a term largely used to describe nanotechnology, refers to synthesis based on atom-by-atom (or molecule-by-molecule) assembly of structures. Silicon microfabrication, such as the methods used to produce the latest generation of computer processors, is still mostly a top-down process, with a minimum feature size of 130 nm, although ongoing advances in lithography will soon permit feature sizes below 100 nm.

In this article, we highlight some of the current micro- and nanotechnologies for drug delivery, with a brief discussion of the background, challenges and recent progress for each method. The discussion is organized by fundamental technologies, rather than by scale. The topics covered include targeted delivery of devices,

[1]Department of Mechanical Engineering, Yale University, New Haven, Connecticut 06520-8284, USA. [2]Polaris Venture Partners, 1000 Winter Street, Waltham, Massachusetts 02451, USA. [3]Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA. Correspondence should be addressed to R.L. (rlanger@mit.edu).

Published online 30 September 2003; doi:10.1038/nbt876

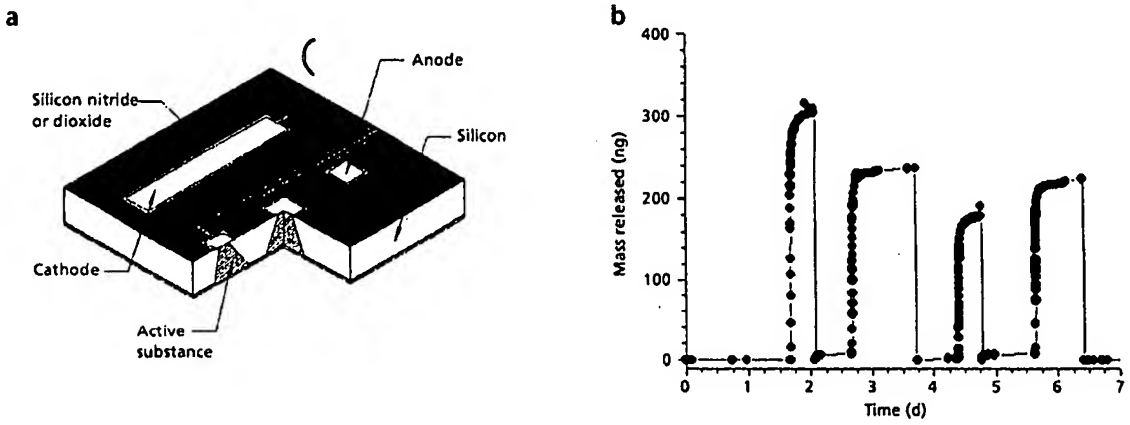

Figure 1 Multiwell silicon-based drug-release device. From ref. 10.

nanoparticles, field activation (remote control) of molecular interactions, diffusion-controlled delivery devices, nanostructured smart surfaces and materials, and the biocompatibility of these devices. Viral vectors are not considered in the brief discussion of gene delivery, which focuses on synthetic methods; nor are microneedles and transdermal methods covered. Particles and liposomes for drug delivery are discussed only in the context of technologies for targeted delivery. Nanotubes and nanowires are also excluded—although they offer unusual capabilities for sensing and delivery, there is as yet relatively little published work in this area related to drug delivery. Several reviews on related topics provide useful perspectives for an interested reader[1–9]. As a long-term goal for small-scale drug delivery technologies is to develop methods that can autonomously treat diseases, we also consider the implications of recent advances in drug administration for future, more 'intelligent' and possibly autonomous systems.

Microfabricated devices

Many types of implantable controlled delivery devices are in various stages of production and clinical evaluation. These devices have been designed to release drugs at various dosages and for both intermittent and continuous delivery. They are designed to operate for short periods (days) or for extended periods (~1 year); some can be refilled during use and others are not designed to be refilled.

One type of design incorporates multiple sealed compartments, which are opened on demand to deliver a dose of a drug[10] (Fig. 1). Another approach is to use microscale pumps and valves that meter delivery from a larger-scale reservoir[11]. For each of these approaches, cost, stability, biocompatibility and long-term functionality are all being studied. As these devices are generally made by micromachining, it should not be difficult to add intelligent control systems.

If the devices are small enough to be ingested, then the ability to target them to certain tissue types, such as regions of the intestine, provides an opportunity to improve oral drug delivery. For example, lectin-modified mucoadhesive liposomes bind in high numbers to the wall of the intestine[12]. Other types of polymer particles have also been made to target drugs to the intestinal wall[13,14]. This approach has been extended to small-scale devices in the shape of free-floating drug delivery 'patches' that adhere to the mucosal membrane in the intestine, shielding the drug from luminal proteolytic enzymes[15]. A similar method uses discs fabricated from carboxymethylcellulose that are coated on several sides with a less permeable ethylcellulose to deliver model drugs by adhering to the mucosal membrane in the intestine, thereby protecting the drug from degradation during absorption[16].

To consider injecting or otherwise targeting micromachined devices for drug delivery in the body, they must be smaller than the thickness of most silicon wafers (~500 mm). The scale of current microdevices produced with integrated circuit fabrication techniques is limited by the thickness of the wafer they are made on. To overcome this barrier, 'off-wafer' methods are being developed in which very small (10–100 μm) devices are made on wafers and then released to float freely by dissolving a 'sacrificial' layer. This approach is intriguing because the current capabilities of top-down micromachining are used to break through the size barrier inherent in wafer thickness. Self-assembled, or bottom-up, devices have not yet shown the same level of functionality. As their scale decreases, drug delivery devices may be delivered by ingestion (~1 mm), injected into tissue (<200 μm), inhaled (<100 μm) or even released into circulation (<10 μm). One example is seen in off-wafer devices (~100 μm) that have been micromachined from poly(methylmethacrylate) (PMMA) to create mucoadhesive particles containing a small well designed to carry a drug payload to the wall of the intestine[17].

Although it is difficult to control the delivery rate of compounds given orally, whether in conventional formulations or in the newer mucoadhesive devices, it has been shown that liposomes containing iron oxide nanoparticles bind with higher affinity to the Peyer's patches in the intestinal wall when administered along with an external magnetic field[18]. This type of approach may provide a way of controlling the mean clearance times of particles or devices, and thereby the amount of drug absorbed.

A more fundamental approach to *in vivo* drug delivery would be on-demand synthesis of a desired molecule. This type of device, though farther from application, has great flexibility in that therapeutics (such as proteins or peptides) could be synthesized as needed, either in a scaled-down version of a chemical synthesizer or by 'programming' captive cells with the appropriate DNA to generate the compound of interest. Programmable systems for chemical synthesis on demand may incorporate advances in microfluidics. Microfluidic devices have been developed for tissue engineering[19] and drug delivery[8]. These systems benefit from reliable valves and pumps[20]. Complex multilevel devices capable of carrying out 144 parallel reactions to explore protein

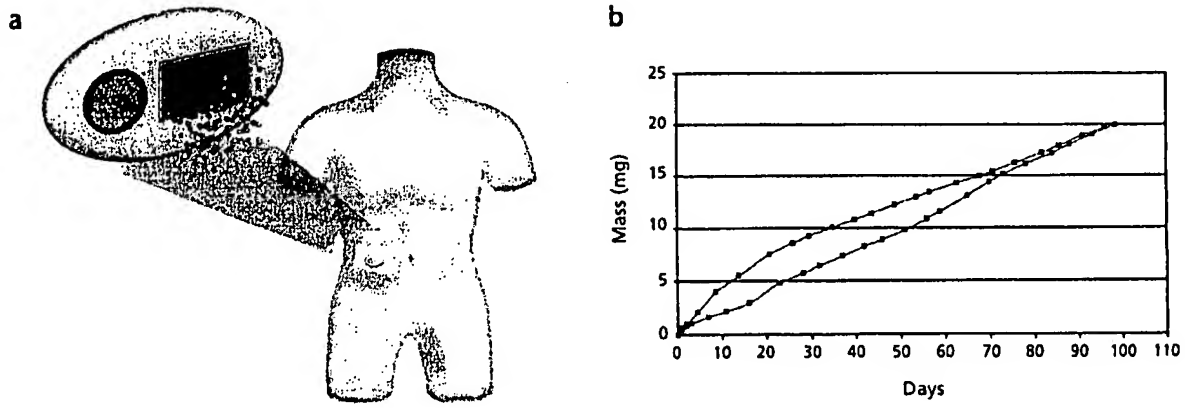

Figure 2 Results of *in vitro* release with 30 mg of a drug in powder form over 100 days (graph courtesy of Debiotech).

crystallization for drug development have been demonstrated[21] and provide a glimpse of the potential of this approach. For example, such systems promise precise control of the crystal structure and hence the bioavailability of the synthesized drug.

Poly(dimethylsiloxane) (PDMS) is a common material for the production of soft microfluidic devices[22,23]. This material is easy to work with, and it is rubbery, which is a good property for many *in vivo* applications. However, it has low toughness, which can make it difficult to handle, and it lacks biodegradability, a property which can be of interest for drug delivery. Other elastomers[24] may be well suited for the development of degradable microfluidic devices. Of course, rigid materials such as silicon[25] and glass[26] have been in use for several years, and nondegradable devices coupling soft and rigid materials have also been designed[27,28].

Chambers

Diffusion chambers holding a cargo of drugs or cells and sealed with a semipermeable membrane have been used as research tools for more than 70 years[29–31]. Micro- and nanofabricated membranes in these devices allow greater control of the dose profile and, in the case of nanoporous membranes, permit the suppression of aspects of the immune response. In addition, the microfabricated devices can include circuitry to control or measure the dose rate and/or other conditions inside the chamber. Whereas the earliest approaches relied on filter membranes with pore sizes of 0.4 μm or larger, newer work has led to devices with pore sizes as small as 20 nm (ref. 32); such systems are discussed further below.

Drug-releasing chambers. A diffusion device with a semipermeable membrane to control the release rate (diffusion rate) from the chamber[33] is shown in Figure 2. Some diffusion chambers are designed to deliver more than one drug[34]. The utility of diffusion chamber–based release methods has been demonstrated for a wide range of diseases, including diabetes[33,35] and cancer[36], and for a range of target sites, including the inner ear[37], spinal cord[38], eye[39] and brain[40].

Potent drugs can be delivered for extended periods using diffusion-controlled implanted tubes. Unlike diffusion chambers, which have a large membrane surface area compared with the reservoir volume for fairly fast release rates, tubes rely on a narrow aperture to provide a slow delivery rate and are usually designed for long-term release of highly potent drugs, with release times on the order of years. Five-year-duration birth control implants based on elastomeric tubes are a notable example of this approach[41]. A newer approach using a titanium tube implant to deliver leuprolide acetate over a period of 12 months[42] has also recently been approved (Fig. 3).

Cell chambers. *In vivo* cell chambers offer a method for using isolated colonies of cells in research and in drug delivery[43]. Xenografted and genetically engineered cells can manufacture therapeutic compounds within the chamber while the chamber keeps the cells physically isolated from the rest of the body and its immune system. Cell chambers have been used to produce compounds such as erythropoietin[44], insulin[45,46] and interferon α[47]. They have also been used to contain cancer cells to stimulate natural cancer-fighting mechanisms[48]. Although such systems have been effective in partitioning cells away from deleterious host cellular immune responses, they often have not excluded humoral immune system components, such as IgG[49]. In this respect, finer membranes (with 100-nm pore size) that reduce access of some immune system components were an improvement over the earlier devices[29]. More recently, micromachined membranes with controlled pore sizes of ~10 nm have been shown to exclude undesirable immune complexes more effectively[32] while permitting reasonably fast release of the desired compound from the chamber.

Another issue in the development of implantable cell chambers concerns the supply of adequate nutrients to the encapsulated cells[50]. One approach to address this is hybrid chambers that supply oxygen by electrolysis of water[51]. Another approach is to generate oxygen by electrolysis and to remove the unwanted protons produced by ion exchange[52].

Nanoparticles

Nanoparticles are already in use in several areas of drug delivery and cosmetics. Usually smaller than 100 nm, they are made by forming nanocrystals or drug-polymer complexes[53] or by creating nanoscale shells (such as liposomes) that entrap drug molecules. Nanoparticles have unusual properties that can be exploited to improve drug delivery. Because of their fine size, they are often taken up by cells where larger particles would be excluded or cleared from the body. Small molecules, peptides, proteins and nucleic acids can be loaded into nanoparticles that are not recognized by the immune system[54] and that can be targeted to particular tissue types. Recent strategies include the use of poly(ethylene glycol) (PEG) to increase circulation time[55] as well as the use of PEG in competition with binding groups to reduce nonspecific attachment or uptake[56]. Multiple targeting

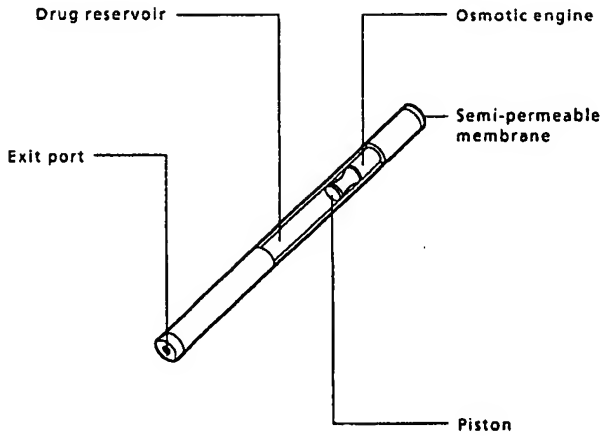

Figure 3 Twelve-month-release leuprolide acetate implant, 4 mm in diameter by 45 mm long. The titanium implant holds 74 mg. (Illustration courtesy of Alza Corp.)

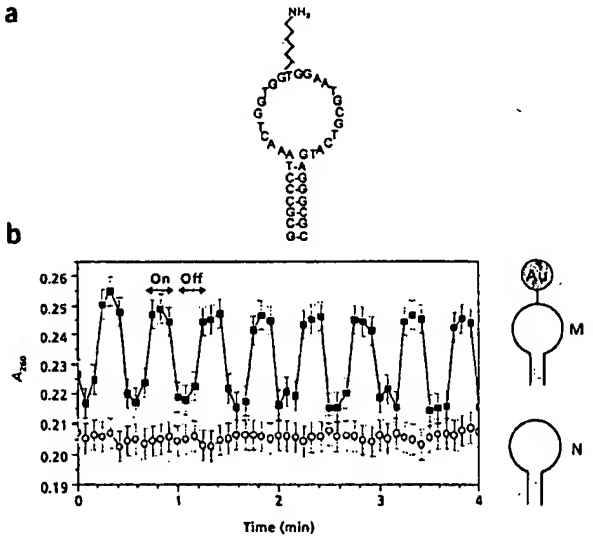

Figure 4 RF activation of DNA hybridization. (a) Molecular structure. (b) Optical absorbance at 260 nm measured as DNA-nanoparticle conjugate and a bare DNA construct are exposed to RF field. From ref. 88.

moieties have been coupled to individual particles, such as the combination of a receptor-mediated endocytosis peptide and a nuclear localization signal peptide for two-step nuclear delivery[57]. Another new strategy is to target liposomes and nanoparticles to short-lived targets, such as radiation-induced neo-antigens formed in tumors during irradiation treatment[58].

Aerosol delivery is a way to carry particles into the deep tissue of the lungs where they are quickly absorbed. Optimizing the size and density of the particles improves delivery efficiency[59,60]. Advantages of aerosol delivery include elimination of the discomfort and stigma of frequent injections. Recent work has shown that nanoparticles can be incorporated into micron-sized, porous carrier particles[61] for aerosol delivery, combining the ease of aerosol delivery and the bioavailability of the nanoparticles released from the larger particle in the deep lung tissue.

Much effort has gone into developing polymeric nanoparticles[62] and liposomes for the delivery of genes, as well as other nonviral gene delivery methods such as the gene gun[63]. Synthetic vectors present promising alternatives to viral delivery for economic, manufacturing and safety reasons[64]. One example is pH-sensitive nanoparticles that remain intact until they have been taken up by a cell, and then, under low-pH conditions, quickly degrade and release their payload[65–67]. Cationic polymers that are salt and serum stable and have bioactive functionalities to enhance intracellular trafficking offer increased stability and delivery efficiency[68]. Recent work that has compared libraries of unique degradable polymers found many that transfected more efficiently than conventional systems, such as poly(ethyleneimine)[69]. Other work has focused on modifying poly(ethyleneimine) to improve transfection efficiency and reduce the toxicity[70]. Another approach is to complex plasmid DNA with stearyl-poly(L-lysine) and low-density lipoprotein. This triplex transfects more efficiently, with longer-duration protein expression, than naked plasmid alone[71].

Another focus of research is the fabrication of metal and metal oxide nanoparticles with antimicrobial activity. Metal ions have been in use for some time as antimicrobial agents: mercury was used, ineffectively, against the Black Plague in Europe, and arsenical compounds were used, more effectively, against syphilis at the turn of the 20th century. The use of antimicrobial formulations containing zinc, cadmium, zirconium or tin salts mixed with polymers dates back to the 1960s[72]. Polymer particles containing metal[73,74] were made in the early 1990s and, more recently, there has been much work on metal-oxide[75] and silver nanoparticles[76,77] formed either from solution phase[77] or *in situ* on a surface[76]. Early work had shown that the effectiveness of silver against bacteria depends on it being both available and in a soluble form[78,79]. A comparison of a silver salt (silver nitrate) and a silver chelating agent (silver sulfadiazine[80]) revealed that they are equally effective[79] and are both significantly more effective than silver ions formed electrochemically[78], which are believed to be not particularly bioavailable. It is interesting to note, however, that there have been reports of silver-resistant *Escherichia coli* infections[81]. Although the antimicrobial agents may be new, the lessons of antibiotic resistant infections should not be ignored.

Strategies for making 'smart' devices

Smart delivery systems allow real-time control of drug dosage according to alterations in chemical and physiological status. In general, two main approaches are used to control the delivery of a drug to a target tissue: activation of molecular interactions using light, radiofrequency (RF) or ultrasound energy, and systems comprising materials with release kinetics that can be modified by an external stimulus.

Activation of molecular interactions. Strategies to control drug delivery by activating molecular interactions on demand have used various external energy sources, such as light, RF or ultrasound. For some time, RF- and ultrasound-mediated local heating (hyperthermia) has been studied as a means of treating cancer[82,83]. Recent work has focused on miniature ferromagnetic seeds[84,85] and rods[86] that can be injected into a tumor to concentrate the heating effect with minimal discomfort. Ferromagnetic materials are chosen with a Curie temperature slightly above body temperature so that these devices have a self-regulating behavior. Ferromagnetic microparticles[87] and superparamagnetic nanoparticles[84] have been developed to target particular tissue types and can be used not only as a means of targeting thermal treatment, but also as a magnetic resonance imaging contrast agent.

Box 1 Biocompatibility

There is a growing body of knowledge about the biocompatibility of small-scale drug delivery devices and materials[130–136]. The literature offers many lessons in how to reduce, and potentially avoid, adverse immune responses. There is also an extensive body of knowledge concerning macroscale implant materials in the form of pacemakers, artificial hips and knees, and other implantable medical devices[137,138]. A search engine administered by the US Food and Drug Administration (FDA; Rockville, Maryland, USA) links to a database of adopted consensus standards on testing methods and materials for macroscale implants, and is a good starting point for questions relating to biocompatibility (www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfStandards/search.cfm). The US Environmental Protection Agency (EPA; Washington, DC) is currently soliciting proposals to evaluate the impact of manufactured nanomaterials on human health and the environment; these projects should provide additional insight into this issue.

Current implants (*e.g.*, pacemakers) are encased in a liquid-tight housing that isolates the mechanisms, batteries and so forth from the *in vivo* environment. In these devices, most working parts are protected from contact with body fluids. In contrast to macroscale devices, many micro- and nanoscale devices are designed as off-water or bare-die devices (devices on the wafer they were fabricated on, without additional packaging) to maintain a small implant size. Bare-die devices raise new issues because the common materials used in their microfabrication have not been used for applications in which they are wetted by body fluids. Several studies assessing reactions to implanted bare-die silicon devices have concluded, however, that silicon and related materials have adequate biocompatibility[132,134–136].

These studies also raise concerns about material stability, such as film delamination[136] and the dissolution of protective layers of gold[135] and silicon oxide (SiO_2)[134]. Recent work has focused on characterizing tissue reactions to 500-μm-thick, 5 mm × 5 mm bare-die and coated samples implanted for periods up to 6 months. A fibrous reaction is expected to encapsulate foreign implants.

Figure 5 Tetrahedral amorphous carbon–coated bare-die implant 5 mm × 5 mm × 0.5 mm thick. (a) After 6 months *in vivo*. (b) Cross-section of capsule formed around implant after 56 days—the 0.5-mm-thick die was removed before sectioning. The thickness of the capsule remained essentially unchanged from this time point to the end of the study at 6 months. The capsules were well vascularized with new blood vessels; a typical blood vessel is marked with an arrow (D. LaVan, D. Kohane, R. Padera, T. Friedmann, J. Sullivan and R. Langer, unpublished data).

Preliminary results show that capsule growth stabilizes after 2 months and the capsules are well vascularized (Fig. 5), allaying many concerns about mass transport that might arise if they were dense and avascular.

One simple method to improve the biocompatibility of a drug delivery device is to modify the surface molecular interactions. Modification with hydrophilic agents[139,140], such as PEG, reduces the surface adsorption of proteins such as fibrinogen, albumin or thrombin[141]. This same approach has been used to modify the *in vivo* behavior of drugs and drug delivery particles. For example, PEG prolongs the circulation times of liposomes[142] and imparts 'stealthiness' to nanoparticles, reducing clearance by the reticuloendothelial system[55]. At present there are at least 18 polymer-protein and polymer-drug complexes in clinical trials, or on the market, in the United States and Europe[143]. Coating with a range of drugs and materials, including anti-inflammatory agents such as dexamethasone[124,125,144], to modify the response of implanted devices is now being investigated.

Localized heating may also be used to modify the release kinetics of locally delivered or targeted drug particles, thus amplifying the benefit of the thermal treatment. Recent work at the molecular scale has shown that coupling of nucleic acids[88] to nanoparticle 'antennas' in a high-frequency RF field can induce localized heating (essentially of only those molecules in intimate contact with the nanoparticle) capable of opening nucleic acid conjugates or activating proteins (Fig. 4)[89]. The ability to use an externally applied RF field to change the conformation of DNA opens the possibility that these nanosystems could be used to regulate mRNA and consequently control protein production on demand. Similarly, changing the activity of an enzyme with an RF field could permit the inhibition, or increase the production, of other proteins. The challenge remains to perfect these methods for *in vivo* use.

Smart surfaces and materials. Controlled release of drugs from degradable polymers is well known, and newer classes of materials are under development, including some whose release rate can be changed *in vivo*. The latter offer opportunities for designing programmable release devices if the release kinetics can be modified by an external stimulus. One such class of materials is hydrogels, polymers that form three-dimensional, highly hydrophilic networks that can incorporate compounds within the permeable structure. When exposed to water, hydrogels swell but do not dissolve. Their ability to absorb and release drugs can be tailored by modifying their molecular design. Several types of hydrogels have been reported, including those that are sensitive to specific antigens[90], are formed from peptide structures[91,92], dissolve with an applied charge[93] or reversibly swell with an applied charge[94]. Each of these properties provides a way of regulating the rate of drug release from the hydrogel matrix or from a reservoir or channel covered by the hydrogel.

A useful feature of some hydrogels is their reversibility, which can be exploited in the form of an implant whose permeability changes in response to the local environment without an external control system. Although hydrogels are not programmable, they do serve to link sensing with drug delivery. Currently, the most direct means of doing this seems to be hydrogels that swell or shrink after exposure to enzymes[95] or antigens[90].

Another technology that is likely to advance the development of programmable, or feedback-controlled, *in vivo* drug delivery devices is nanostructured 'smart' surfaces. Combining a smart surface or

membrane with an otherwise diffusion-controlled delivery device permits the release rate to be regulated by changing the permeability of the membrane. Switchable surfaces and membranes can be controlled by light[96,97], heat[98,99], pH[100,101] and redox and amperometric reactions[102]. For some of the materials, the state (or phase) is reversible, whereas for others it is set once. Drug delivery devices that allow control of the dose rate have been based on electrophoresis[103], diffusion-controlled hydrogels[94,104,105] and thermally switchable materials[106,107]. An electrically controllable surface would provide a direct method to switch release rates. A recently described method uses 1-nm-tall self-assembled monolayers that change conformation with charge and, as a result, reversibly change from a hydrophobic to hydrophilic state with small alterations in charge[108,109].

Coupling drug delivery to sensors and other implants

One of the long-term goals for *in vivo* drug delivery is to couple smart drug delivery devices to other implants, such as biosensors, pacemakers and stents. To date, a limiting step in the creation of feedback-controlled drug delivery systems has been the development of stable sensors. Many of the early projects to integrate drug delivery with sensing focused on the treatment of diabetes, with systems to sense blood glucose levels and release insulin in response[110]. Unfortunately, no fully automatic long-term *in vivo* system has been brought to market because of stability problems with *in vivo* glucose sensors[111]. Other types of electrochemical sensors, such as ones to measure mixed venous oxygen pressure, have functioned *in vivo* for as long as 4 years[112].

An ideal *in vivo* drug delivery system would be able to determine when and if a dose was needed and then deliver it automatically. To do this, sensors are needed to monitor physical or biochemical conditions. Robust RF pressure sensors based on battery-powered designs that can function *in vivo* for 4–6 months have been used for several years[113]. Other approaches to *in vivo* sensing are also based on devices that rely on energy harvested from remote sources. Remotely powered and passive sensors can operate for extended periods (indefinitely, if evaluated solely on the basis of power) and can be made smaller than battery-powered devices. Several types of wireless or passive sensors that harvest RF energy[114–120] are being developed for applications such as measuring intraocular[116], intracranial and arterial[117,119] pressure.

RF is not the only source of energy available; another *in vivo* pressure sensor uses energy harvested from ultrasound[121], which penetrates tissue more deeply than does high-frequency RF. Another alternative to RF is *in vivo* energy generation by a glucose-driven fuel cell[122], which offers the potential for very high energy densities. Batteryless and wireless sensing is a good way to close the loop with drug delivery, as the energy harvested to operate the sensor could just as easily be applied to operate a drug delivery device, and the lack of batteries means that these devices could function for extended periods and have fewer safety concerns.

The coupling of drug delivery to sensors is only one aspect of the linkage of *in vivo* drug delivery to hardware. There are many medical devices implanted each year, and each of them could be viewed as a potential platform for providing local drug delivery. Although not based on micro- or nanotechnologies, drug-coated stents seem to be very effective in delivering drugs directly at the site of implantation[123–128] to substantially reduce the risk of restenosis.

Conclusions

Although some of the technologies described above, such as cosmetic formulations with nanoparticles and several diffusion-based delivery devices, are in use or are being evaluated in clinical trials, new materials and the devices based on them will require thorough testing to evaluate both safety and efficacy before clinical use (see Box 1). Micro- and nanotechnologies are changing the way drug delivery is being approached, offering new ways to deliver drugs effectively and to reduce overall dosage (and side effects).

One of the main goals of drug delivery will be to more efficiently target therapies to specific tissue types. This will increase drug efficacy by sequestering a drug where it is needed and also ensure that healthy tissues are spared. To accomplish this goal, further work is needed to verify that devices deliver drugs to the desired tissue types across large populations of patients.

'On demand' or programmable drug delivery methods offer an opportunity to create fully autonomous systems, which, when coupled with sensors, could measure local concentrations of biomarkers or drugs and release the appropriate compound in response. Simple closed-loop delivery systems that measure glucose levels and release insulin to treat diabetes are currently being evaluated in clinical trials for short periods (2–3 days)[129]; new algorithms and methods are needed, however, as there is little experience with real-time control of drug delivery. These projects should help provide the systems integration and control algorithms that will be an important step toward fully autonomous drug administration.

ACKNOWLEDGMENTS

D.L. and R.L. thank the US National Institutes of Health for support.

Published online at http://www.nature.com/naturebiotechnology/

1. Langer, R. New methods of drug delivery. *Science* 249, 1527–1533 (1990).
2. Reed, M.L. *et al.* Micromechanical devices for intravascular drug delivery. *J. Pharm. Sci.* 87, 1387–1394 (1998).
3. Dario, P., Carrozza, M.C., Benvenuto, A. & Menciassi, A. Micro-systems in biomedical applications. *J. Micromech. Microeng.* 10, 235–244 (2000).
4. Polla, D.L. *et al.* Microdevices in medicine. *Annu. Rev. Biomed. Eng.* 2, 551–576 (2000).
5. Lavan, D.A., Lynn, D.,M. & Langer, R. Moving smaller in drug discovery and delivery. *Nat. Rev. Drug. Discov.* 1, 77–84 (2002).
6. Shawgo, R.S., Richards Grayson, A.C., Li, Y. & Cima, M.J. BioMEMS for drug delivery. *Curr. Opin. Solid State Mater. Sci.* 6, 329–334 (2002).
7. Tao, S.L. & Desai, T.A. Microfabricated drug delivery systems: from particles to pores. *Adv. Drug Deliv. Rev.* 55, 315–328 (2003).
8. Saltzman, W.M. & Olbricht, W.L. Building drug delivery into tissue engineering. *Nat. Rev. Drug Discov.* 1, 177–186 (2002).
9. McAllister, D.V., Allen, M.G. & Prausnitz, M.R. Microfabricated microneedles for gene and drug delivery. *Annu. Rev. Biomed. Eng.* 2, 289–313 (2000).
10. Santini, J.T. Jr., Cima, M.J. & Langer, R. A controlled-release microchip. *Nature* 397, 335–338 (1999).
11. Maillefer, D., Van Lintel, H., Rey-Mermet, G. & Hirschi, R. A high-performance silicon micropump for an implantable drug delivery system. in *12th IEEE International Conference on Micro Electro Mechanical Systems, Technical Digest, Orlando, Florida, USA, Jan 17–21, 1999* 541–546 (IEEE, New York, USA, 1999).
12. Chen, H., Torchilin, V. & Langer, R. Lectin-bearing polymerized liposomes as potential oral vaccine carriers. *Pharm. Res.* 13, 1378–1383 (1996).
13. Woodley, J. Bioadhesion: new possibilities for drug administration? *Clin. Pharmacokinet.* 40, 77–84 (2001).
14. Keegan, M.E., Whittum-Hudson, J.A. & Saltzman, M.W. Biomimetic design in microparticulate vaccines. *Biomaterials* 24, 4435–4443 (2003).
15. Ito, Y., Hu, Z., Yoshikawa, Y., Murakami, M. & Takada, K. Oral bioadhesive patch system for the delivery of peptide/proteins. in *Proceedings of the 26th International Symposium on Controlled Release of Bioactive Materials* pp. 851–852 (Controlled Release Society, Minneapolis, Minnesota, USA, 1999).
16. Shen, Z. & Mitragotri, S. Intestinal patches for oral drug delivery. *Pharm. Res.* 19, 391–395 (2002).
17. Tao, S.L., Lubeley, M.W. & Desai, T.A. Bioadhesive poly(methyl methacrylate) microdevices for controlled drug delivery. *J. Control. Release* 88, 215–228 (2003).
18. Chen, H. & Langer, R. Magnetically-responsive polymerized liposomes as potential oral delivery vehicles. *Pharm. Res.* 14, 537–540 (1997).
19. Borenstein, J.T., King, K.R., Terai, H. & Vacanti, J.P. Capillary formation in microfabricated polymer scaffolds. *Mater. Res. Soc. Symp. Proc.* 711, 163–168 (2002).
20. Unger, M.A., Chou, H.-P., Thorsen, T., Scherer, A. & Quake, S.R. Monolithic microfabricated valves and pumps by multilayer soft lithography. *Science* 288, 113–116 (2000).
21. Hansen, C.L., Skordalakes, E., Berger, J.M. & Quake, S.R. A robust and scalable microfluidic metering method that allows protein crystal growth by free interface diffusion. *Proc. Natl. Acad. Sci. USA* 99, 16531–16536 (2002).

22. Xia, Y. & Whitesides, G.M. Soft lithography. *Polym. Mater. Sci. Eng.* **77**, 596–598 (1997).
23. Quake, S.R. & Scherer, A. From micro- to nanofabrication with soft materials. *Science* **290**, 1536–1540 (2000).
24. Wang, Y., Ameer, G.A., Sheppard, B.J. & Langer, R. A tough biodegradable elastomer. *Nat. Biotechnol.* **20**, 602–606 (2002).
25. Manz, A. *et al.* Micromachining of monocrystalline silicon and glass for chemical analysis systems. A look into next century's technology or just a fashionable craze? *Trends Anal. Chem.* **10**, 144–149 (1991).
26. Harrison, D.J., Manz, A., Fan, Z., Luedi, H. & Widmer, H.M. Capillary electrophoresis and sample injection systems integrated on a planar glass chip. *Anal. Chem.* **64**, 1926–1932 (1992).
27. Goll, C. *et al.* Microvalves with bistable buckled polymer diaphragms. *J. Micromech. Microeng.* **6**, 77–79 (1996).
28. Schomburg, W.K., Buestgens, B., Fahrenberg, J. & Maas, D. Components for microfluidic handling modules. in *Proceedings of μTAS '94: The 1st Workshop on Micro Total Analytical Systems* (ed. Bergveld, P.) pp. 255–258 (Kluwer, Dordrecht, The Netherlands, 1995).
29. Ohgawara, H., Hirotani, S., Miyazaki, J. & Teraoka, S. Membrane immunoisolation of a diffusion chamber for bioartificial pancreas. *Artif. Organs* **22**, 788–794 (1998).
30. Colton, C.K. Implantable biohybrid artificial organs. *Cell Transplant.* **4**, 415–436 (1995).
31. Desai, T.A., Chu, W.H., Tu, J., Shrewsbury, P. & Ferrari, M. Microfabricated biocapsules for cell xenografts: a review. *Proc. SPIE* **2978**, 216–226 (1997).
32. Desai, T.A. *et al.* Microfabricated immunoisolating biocapsules. *Biotechnol. Bioeng.* **57**, 118–120 (1998).
33. Sefton, M.V., Horvath, V. & Zingg, W. Insulin delivery by a diffusion-controlled micropump in pancreatectomized dogs: phase 1. *J. Control. Release* **12**, 1–12 (1990).
34. Atkinson, L.E., Dunn, J.T., Gale, R.M. & Rivera, D.L. Segmented device for simultaneous delivery of multiple beneficial agents. US Patent 5443461 (1995).
35. Hanas, R. Selection for and initiation of continuous subcutaneous insulin infusion. *Horm. Res.* **57**, 101–104 (2002).
36. Damascelli, B. *et al.* Circadian continuous chemotherapy of renal cell carcinoma with an implantable, programmable infusion pump. *Cancer* **66**, 237–241 (1990).
37. Brown, J.N., Miller, J.M., Altschuler, R.A. & Nuttall, A.L. Osmotic pump implant for chronic infusion of drugs into the inner ear. *Hearing Res.* **70**, 167–172 (1993).
38. Gardner, B. *et al.* Intrathecal baclofen—a multicentre clinical comparison of the Medtronics Programmable, Cordis Secor and Constant Infusion Infusaid drug delivery systems. *Paraplegia* **33**, 551–554 (1995).
39. Blair, M.J. *et al.* Subconjunctivally implanted micro-osmotic pumps for continuous ocular treatment in horses. *Am. J. Veter. Res.* **60**, 1102–1105 (1999).
40. Fisher, R.S. & Ho, J. Potential new methods for antiepileptic drug delivery. *CNS Drugs* **16**, 579–593 (2002).
41. Diaz, S. *et al.* A five-year clinical trial of levonorgestrel silastic implants (Norplant tm). *Contraception* **25**, 447–456 (1982).
42. Wright, J.C. *et al.* A one-year implantable, osmotic delivery system (Duros leuprolide implant) for the treatment of advanced prostate cancer. In *Proceedings of the 24th International Symposium on Controlled Release of Bioactive Materials* 59–60 (Controlled Release Society, Minneapolis, Minnesota, USA, 1997).
43. Ohgawara, H. Strategies for immunoisolation in islet transplantation: challenges for the twenty-first century. *J. Hepato-Biliary-Pancreatic Surg.* **7**, 374–379 (2000).
44. Regulier, E., Schneider, B.L., Deglon, N., Beuzard, Y. & Aebischer, P. Continuous delivery of human and mouse erythropoietin in mice by genetically engineered polymer encapsulated myoblasts. *Gene Ther.* **5**, 1014–1022 (1998).
45. Jolley, W.B., Hinshaw, D.B., Call, T.W. & Alvord, L.S. Xenogeneic pancreatic islet transplantation in proteolytic enzyme–bonded diffusion chambers in diabetic rats. *Transplant. Proc.* **9**, 363–365 (1977).
46. Desai, T.A. *et al.* Microfabricated biocapsules provide short-term immunoisolation of insulinoma xenografts. *Biomed. Microdevices* **1**, 131–138 (1999).
47. Ogura, H. *et al.* Implantation of genetically manipulated fibroblasts into mice as antitumor. Alpha-interferon therapy. *Cancer Res.* **50**, 5102–5106 (1990).
48. Brauker, J.H., Geller, R.L., Johnston, W.D., Levon, S.A. & Maryanov, D.A. Implantation of tumor cells for the prevention and treatment of cancer. US Patent 6156305, Cont.-in-part of U.S. Ser. No. 272,189, abandoned (2000).
49. Brauker, J., Martinson, L.A., Young, S.K. & Johnson, R.C. Local inflammatory response around diffusion chambers containing xenografts. Nonspecific destruction of tissues and decreased local vascularization. *Transplantation* **61**, 1671–1677 (1996).
50. Kuhtreiber, W.M., Lanza, R.P., Beyer, A.M., Kirkland, K.S. & Chick, W.L. Relationship between insulin secretion and oxygen tension in hybrid diffusion chambers. *ASAIO J.* **39**, M247–M251 (1993).
51. Vardi, P., Gross, Y., Bloch, K., Bloch, D. & Boukobza, N. Implantable device comprising oxygen generator. PCT International Application 0150983 (2001).
52. Colton, C.K. & Swette, L.L. Method of delivering oxygen to cells by electrolyzing water. US Patent 6368592 (2002).
53. Cohen, H. *et al.* Sustained delivery and expression of DNA encapsulated in polymeric nanoparticles. *Gene Ther.* **7**, 1896–1905 (2000).
54. Merisko-Liversidge, E. *et al.* Formulation and antitumor activity evaluation of nanocrystalline suspensions of poorly soluble anticancer drugs. *Pharm. Res.* **13**, 272–278 (1996).
55. Gref, R. *et al.* Biodegradable long-circulating polymer nanospheres. *Science* **263**, 1600–1603 (1994).
56. Akerman, M.E., Chan, W.C.W., Laakkonen, P., Bhatia, S.N. & Ruoslahti, E. Nanocrystal targeting *in vivo*. *Proc. Natl. Acad. Sci. USA* **99**, 12617–12621 (2002).
57. Tkachenko Alexander, G. *et al.* Multifunctional gold nanoparticle-peptide complexes for nuclear targeting. *J. Am. Chem. Soc.* **125**, 4700–4701 (2003).
58. Hallahan, D. *et al.* Integrin-mediated targeting of drug delivery to irradiated tumor blood vessels. *Cancer Cell* **3**, 63–74 (2003).
59. Edwards, D.A. *et al.* Large porous particles for pulmonary drug delivery. *Science* **276**, 1868–1871 (1997).
60. Edwards, D.A. & Dunbar, C. Bioengineering of therapeutic aerosols. *Annu. Rev. Biomed. Eng.* **4**, 93–107 (2002).
61. Tsapis, N., Bennett, D., Jackson, B., Weitz, D.A. & Edwards, D.A. Trojan particles: large porous carriers of nanoparticles for drug delivery. *Proc. Natl. Acad. Sci. USA* **99**, 12001–12005 (2002).
62. Luo, D. & Saltzman, W.M. Synthetic DNA delivery systems. *Nat. Biotechnol.* **18**, 33–37 (2000).
63. Yang, N.S., Burkholder, J., Roberts, B., Martinell, B. & McCabe, D. *In vivo* and *in vitro* gene transfer to mammalian somatic cells by particle bombardment. *Proc. Natl. Acad. Sci. USA* **87**, 9568–9572 (1990).
64. Anderson, W.F. Human gene therapy. *Nature* **392**, 25–30 (1998).
65. Lynn, D.M., Amiji, M.M. & Langer, R. pH-responsive polymer microspheres: rapid release of encapsulated material within the range of intracellular ph. *Angew. Chem. Int. Ed. Engl.* **40**, 1707–1710 (2001).
66. De Jaeghere, F. *et al.* pH-dependent dissolving nano- and microparticles for improved peroral delivery of a highly lipophilic compound in dogs. *AAPS Pharm. Sci.* **3**, E8 (2001).
67. Choi, J.S., Mackay, J.A. & Szoka, F.C. Jr. Low-pH-sensitive PEG-stabilized plasmid-lipid nanoparticles: preparation and characterization. *Bioconj. Chem.* **14**, 420–429 (2003).
68. Hwang, S.J. & Davis, M.E. Cationic polymers for gene delivery: designs for overcoming barriers to systemic administration. *Curr. Opin. Mol. Ther.* **3**, 183–191 (2001).
69. Anderson, D.G., Lynn, D.M. & Langer, R. Semi-automated synthesis and screening of a large library of degradable cationic polymers for gene delivery. *Angew. Chem. Int. Ed. Engl.* **42**, 3153–3158 (2003).
70. Lee, M., Rentz, J., Han, S.O., Bull, D.A. & Kim, S.W. Water-soluble lipopolymer as an efficient carrier for gene delivery to myocardium. *Gene Ther.* **10**, 585–593 (2003).
71. Affleck, D.G., Yu, L., Bull, D.A., Bailey, S.H. & Kim, S.W. Augmentation of myocardial transfection using TerplexDNA: a novel gene delivery system. *Gene Ther.* **8**, 349–353 (2001).
72. Antibacterial detergent compositions contalning heavy metal salts of 2-pyridinethione-1-oxide. British Patent 1111708 (1968).
73. Kazama, T. & Irie, T. Microbicidal thermoplastic composites. Japanese Patent 03273040 (1991).
74. Sasaki, S., Suzuki, K. & Okuma, S. Antibacterial cellulose microparticles. Japanese Patent 04122743 (1992).
75. Stoimenov, P.K., Klinger, R.L., Marchin, G.L. & Klabunde, K.J. Metal oxide nanoparticles as bactericidal agents. *Langmuir* **18**, 6679–6686 (2002).
76. Davenas, J., Thevenard, P., Philippe, F. & Arnaud, M.N. Surface implantation treatments to prevent infection complications in short term devices. *Biomolec. Eng.* **19**, 263–268 (2002).
77. Lee, H.J., Yeo, S.Y. & Jeong, S.H. Antibacterial effect of nanosized silver colloidal solution on textile fabrics. *J. Mater. Sci.* **38**, 2199–2204 (2003).
78. Berger, T.J., Spadaro, J.A., Chapin, S.E. & Becker, R.O. Electrically generated silver ions: quantitative effects on bacterial and mammalian cells. *Antimicrob. Agents Chemother.* **9**, 357–358 (1976).
79. Modak, S.M. & Fox, C.L. Jr. Binding of silver sulfadiazine to the cellular components of *Pseudomonas aeruginosa*. *Biochem. Pharmacol.* **22**, 2391–2404 (1973).
80. Fox, C.L. Jr., Rappole, B. & Stanford, W. Control of pseudomonas infection in burns by silver sulfadiazine. *Surg. Gynecol. Obstet.* **128**, 1021–1026 (1969).
81. Starodub, M.E. & Trevors, J.T. Silver resistance in *Escherichia coli* r1. *J. Med. Microbiol.* **29**, 101–110 (1989).
82. Chive, M., Nguyen, D.D. & Leroy, Y. Localized hyperthermia. Towards an atraumatic method of heat control based on microwave thermography (author's translation). *Bull. Cancer* **68**, 293–294 (1981).
83. Marchal, C. *et al.* Treatment of superficial cancerous tumors by hyperthermia induced by ultrasonics or microwaves. *C. R. Seances Soc. Biol. Fil.* **177**, 358–367 (1983).
84. Pouliquen, D. Magnetite-dextran nanocapsules: preparation and properties. *Microspheres Microcapsules Liposomes* **3**, 495–523 (2001).
85. Lilly, M.B., Brezovich, I.A. & Atkinson, W.J. Hyperthermia induction with thermally self-regulated ferromagnetic implants. *Radiology* **154**, 243–244 (1985).
86. Tucker, R.D., Huidobro, C., Larson, T. & Platz, C.E. Use of permanent interstitial temperature self-regulating rods for ablation of prostate cancer. *J. Endourology* **14**, 511–517 (2000).
87. Arshady, R. Radiolabeled and magnetic microparticles: Introduction and overview. *Microspheres Microcapsules Liposomes* **3**, 1–37 (2001).
88. Hamad-Schifferli, K., Schwartz, J.J., Santos, A.T., Zhang, S. & Jacobson, J.M. Remote electronic control of DNA hybridization through inductive coupling to an attached metal nanocrystal antenna. *Nature* **415**, 152–155 (2002).
89. Hamad-Schifferli, K., Schwartz, J.J., Santos, A.T., Zhang, S. & Jacobson, J.M. Direct electronic control of biomolecular systems: using nanocrystals as antennas for regulation of biological activity. in *Synthesis, Functional Properties and Applications of Nanostructures*. Materials Research Society Symposium Proceedings vol. 676 (eds. Hahn, H.W., Feldheim, D.L., Kubiak, C.P., Tannenbaum, R. & Siegel, R.W.) (Materials Research Society, Warrendale, Pennsylvania, USA, 2001).
90. Miyata, T., Asami, N. & Uragami, T. A reversibly antigen-responsive hydrogel. *Nature* **399**, 766–769 (1999).

91. Wang, C., Stewart, R.J. & Kopecek, J. Hybrid hydrogels assembled from synthetic polymers and coiled-coil protein domains. *Nature* **397**, 417–420 (1999).
92. Nowak, A.P. *et al.* Rapidly recovering hydrogel scaffolds from self-assembling diblock copolypeptide amphiphiles. *Nature* **417**, 424–428 (2002).
93. Kwon, I.C., Bae, Y.H. & Kim, S.W. Electrically erodible polymer gel for controlled release of drugs. *Nature* **354**, 291–293 (1991).
94. Madou, M.J. & He, K.-Q. Exploitation of a novel artificial muscle for controlled drug delivery. *Polym. Mater. Sci. Eng.* **83**, 495–497 (2000).
95. Heller, J. & Trescony, P.V. Controlled drug release by polymer dissolution. Ii: Enzyme-mediated delivery device. *J. Pharmaceutical Sci.* **68**, 919–921 (1979).
96. Abbott, S., Ralston, J., Reynolds, G. & Hayes, R. Reversible wettability of photoresponsive pyrimidine-coated surfaces. *Langmuir* **15**, 8923–8928 (1999).
97. Ichimura, K., Oh, S.-K. & Nakagawa, M. Light-driven motion of liquids on a photoresponsive surface. *Science* **288**, 1624–1626 (2000).
98. Lin, S.-Y., Lin, Y.-Y. & Chen, K.-S. A thermoswitchable membrane for drug delivery. *Drug Deliv.* **2**, 123–127 (1995).
99. Chen, K.-S., Lin, Y.-Y. & Lin, S.-Y. Thermally on-off switching nylon membrane for controlling drug penetration. *Drug Deliv. Syst.* **11**, 55–61 (1996).
100. Okahata, S., Seki, T. & Yonemori, K. pH-sensitive nylon capsule membranes. Japanese Patent 61025633 (1986).
101. Wilson, M.D. & Whitesides, G.M. The anthranilate amide of 'polyethylene carboxylic acid' shows an exceptionally large change with ph in its wettability by water. *J. Am. Chem. Soc.* **110**, 8718–8719 (1988).
102. Willner, I.I. & Katz, E. Integration of layered redox proteins and conductive supports for bioelectronic applications. *Angew. Chem. Int. Ed. Eng.* **39**, 1180–1218 (2000).
103. Sweedler, J.V., Kuo, T.-C., Cannon, D., Bohn, P.W. & Shannon, M.A. Molecular gates for attoscale preparative separations. in *Abstracts of Papers, 222nd American Chemical Society National Meeting, Chicago, Illinois, USA, August 26–30, 2001* ANYL-162 (American Chemical Society, Washington, DC, 2001).
104. Cao, X., Lai, S. & Lee, L.J. Design of a self-regulated drug delivery device. *Biomed. Microdevices* **3**, 109–117 (2001).
105. Umamaheswari, R.B., Jain, P. & Jain, N.K. Hydrogel—a novel drug delivery system. *Indian Drugs* **39**, 243–256 (2002).
106. Mohr, J.M., Schmitt, E.E. & Stewart, R.F. Pulsatile transdermal drug delivery, in *Proceedings of the 19th International Symposium on Controlled Release of Bioactive Materials* 377–378 (Controlled Release Society, 1992).
107. Kwok, C.S.-K., Ratner, B.D., Mourad, P.D. & Crum, L.A. Polymer-based controlled-release drug delivery devices. US Patent 6444217 (2002).
108. Tran, T.-N. Design of reversible 'smart' surfaces for biomedical and nanotechnological applications. Ph.D. Thesis, Massachusetts Institute of Technology (2003).
109. Lahann, J. *et al.* A reversibly switching surface. *Science* **299**, 371–374 (2003).
110. Nagakura, T., Ishihara, K., Furukawa, T., Masuda, K. & Tsuda, T. Auto-regulated osmotic pump for insulin therapy by sensing glucose concentration without energy supply. *Sens. Actuators B Chem.* **B34**, 229–233 (1996).
111. Kerner, W. Implantable glucose sensors: present status and future developments. *Exp. Clin. Endocrinol. Diabetes* **109**, S341–S346 (2001).
112. Holmstrom, N., Nilsson, P., Carlsten, J. & Bowald, S. Long-term *in vivo* experience of an electrochemical sensor using the potential step technique for measurement of mixed venous oxygen pressure. *Biosens. Bioelectron.* **13**, 1287–1295 (1998).
113. Mills, P.A. *et al.* A new method for measurement of blood pressure, heart rate, and activity in the mouse by radiotelemetry. *J. Appl. Physiol.* **88**, 1537–1544 (2000).
114. Husak, M. One-chip integrated resonance circuit with a capacitive pressure sensor. *J. Micromech. Microeng.* **7**, 173–178 (1997).
115. English, J.M. & Allen, M.G. Wireless micromachined ceramic pressure sensors. in *12th IEEE International Conference on Micro Electro Mechanical Systems, Technical Digest*, Orlando, Florida, USA, January 17–21, 1999 377–378 (IEEE, Piscataway, New Jersey, USA, 1999).
116. Schnakenberg, U. *et al.* Initial investigations on systems for measuring intraocular pressure. *Sens. Actuators A Phys.* **A85**, 287–291 (2000).
117. Schnakenberg, U. *et al.* Transponder system for non-invasive measurement of intravascular pressure. *Biomed. Tech. (Berl.)* **47** (Suppl. 1 Pt. 1), 191–193 (2002).
118. Dehennis, A. & Wise, K.D. A double-sided single-chip wireless pressure sensor. in *15th IEEE International Conference on Micro Electro Mechanical Systems, Technical Digest*, Las Vegas, Nevada, USA, January 20–24, 2002 252–255 (IEEE, Piscataway, New Jersey, USA, 2002).
119. Schmitz-Rode, T. *et al.* Vascular capsule for telemetric monitoring of blood pressure. *Rofo. Fortschr. Geb. Rontgenstr. Neuen Bildgeb. Verfahr.* **175**, 282–286 (2003).
120. Allen, M.G. & English, J.M. System, method, and sensors for sensing physical properties. US Patent 6278379, Cont.-in-part of Ser. No. US 1998-54011, filed on 54012 Apr 51998, now (2001).
121. Porat, Y., Penner, A. & Doron, E. Implantable acoustic biosensing system and method. US Patent 6432050 (2002).
122. Mano, N., Mao, F. & Heller, A. Characteristics of a miniature compartment-less glucose-O_2 biofuel cell and its operation in a living plant. *J. Am. Chem. Soc.* **125**, 6588–6594 (2003).
123. Lambert, T.L. *et al.* Localized arterial wall drug delivery from a polymer-coated removable metallic stent: kinetics, distribution, and bioactivity of forskolin. *Circulation* **90**, 1003–1011 (1994).
124. Lincoff, A.M., Furst, J.G., Ellis, S.G., Tuch, R.J. & Topol, E.J. Sustained local delivery of dexamethasone by a novel intravascular eluting stent to prevent restenosis in the porcine coronary injury model. *J. Am. Coll. Cardiol.* **29**, 808–816 (1997).
125. Strecker, E.P. *et al.* Effect on intimal hyperplasia of dexamethasone released from coated metal stents compared with non-coated stents in canine femoral arteries. *Cardiovasc. Intervent. Radiol.* **21**, 487–496 (1998).
126. Ahn, Y.K. *et al.* Preventive effects of the heparin-coated stent on restenosis in the porcine model. *Catheter. Cardiovasc. Interv.* **48**, 324–330 (1999).
127. Grube, E. & Bullesfeld, L. Initial experience with paclitaxel-coated stents. *J. Interv. Cardiol.* **15**, 471–475 (2002).
128. Sonoda, S. *et al.* Taxol-based eluting stents from theory to human validation: clinical and intravascular ultrasound observations. *J. Invasive Cardiol.* **15**, 109–114 (2003).
129. Renard, E., Costalat, G. & Bringer, J. From external to implantable insulin pump, can we close the loop? *Diabetes Metab.* **28**, 2S19–12S25 (2002).
130. Davis, S.D. *et al.* Biocompatibility of ceramic implants in soft tissue. *J. Biomed. Mater. Res.* **6**, 425–449 (1972).
131. Herzog, V., Wolf, A., Glauche, R. & Rueckert, D. Coating of polymeric medical implants with silicon nitride or silicon oxide. East German Patent 282391 (1990).
132. Desai, T.A., Ferrari, M. & Mazzoni, G. Silicon microimplants: fabrication and biocompatibility. *Mater. Design Technol.* **71**, 97–103 (1995).
133. Hernandez, P.R. *et al.* Evaluation of biocompatibility of pH-ISFET materials during long-term subcutaneous implantation. *Sens. Actuators B Chem.* **B46**, 133–138 (1998).
134. Hammerle, H. *et al.* Biostability of micro-photodiode arrays for subretinal implantation. *Biomaterials* **23**, 797–804 (2001).
135. Chow, A.Y. *et al.* Implantation of silicon chip microphotodiode arrays into the cat subretinal space. *IEEE Trans. Neural. Syst. Rehabil. Eng.* **9**, 86–95 (2001).
136. Voskerician, G. *et al.* Biocompatibility and biofouling of MEMS drug delivery devices. *Biomaterials* **24**, 1959–1967 (2003).
137. Von Recum, A. *Handbook of Biomaterials Evaluation: Scientific, Technical, and Clinical Testing of Implant Materials* edn. 2 (Taylor & Francis, Philadelphia, USA, 1999).
138. Dee, K.C., Puleo, D.A. & Bizios, R. *An Introduction to Tissue–Biomaterial Interactions* (Wiley, Hoboken, New Jersey, USA, 2002).
139. Allen, T.M. Stealth liposomes: five years on. *J. Liposome Res.* **2**, 289–305 (1992).
140. Langer, R. Drug delivery and targeting. *Nature* **392**, 5–10 (1998).
141. Wasiewski, W., Fasco, M.J., Martin, B.M., Detwiler, T.C. & Fenton, J.W. Thrombin adsorption to surfaces and prevention with polyethylene glycol 6,000. *Thrombosis Res.* **8**, 881–886 (1976).
142. Blume, G. & Cevc, G. Liposomes for the sustained drug release *in vivo*. *Biochim. Biophys. Acta* **1029**, 91–97 (1990).
143. Duncan, R. The dawning era of polymer therapeutics. *Nat. Rev. Drug Discov.* **2**, 347–360 (2003).
144. Shain, W. *et al.* Controlling cellular reactive responses around neural prosthetic devices using peripheral and local intervention strategies. *IEEE Trans. Neural Syst. Rehabil. Eng.* **11**, 186–188 (2003).

Expert Opinion

1. Introduction
2. Mechanism of action and resistance
3. Pharmacokinetics
4. Efficacy and support studies
5. Expanding therapeutic indications
6. Safety and tolerability
7. Expert opinion

Ashley Publications
www.ashley-pub.com

Overview of the use of the anti-TNF agent infliximab in chronic inflammatory diseases

Jürgen Braun[†] & Joachim Sieper
[†]*Rheumazentrum Ruhrgebiet, Landgrafenstr. 15, 44652 Herne, Department of Rheumatology, Klinikum B Franklin, Free Berlin, Germany*

Anti-inflammatory therapy with monoclonal antibodies (mAbs) directed against tumour necrosis factor (TNF)-α has emerged as a major advancement in the treatment of various immune mediated diseases such as rheumatoid arthritis, ankylosing spondylitis, psoriatic arthritis and Crohn's disease. TNF-α seems to play a major pathogenic role in these chronic immune-mediated inflammatory diseases. Infliximab (Remicade®, Centocor, Inc., Malvern, PA, USA), a chimaeric mAb, binds to soluble and membrane bound TNF-α, but not to TNF-β. Infliximab is able to effectively regulate and mediate inflammatory processes involved in a number of different disease states. Many clinical trials in these diseases have demonstrated that biological therapy with mAbs directed against TNF-α is effective and relatively safe.

Keywords: anti-TNF-α therapy, infliximab, monoclonal antibodies

Expert Opin. Biol. Ther. (2003) 3(1):141-168

1. Introduction

The pathogenic cause of most chronic inflammatory diseases is not known, but the immune system is widely believed to play a major role. The diseases covered by the term "immune-mediated inflammatory diseases" are certainly heterogeneous, but it makes even more sense than in earlier days to use the term because of the central role of tumour necrosis factor (TNF)-α for immune responses. Several biologics are known to inhibit TNF-α. Infliximab has been the first monoclonal antibody (mAb) produced with that ability. This review concentrates on the efficacy and safety of infliximab in some of the most frequent chronic inflammatory diseases: rheumatoid arthritis (RA), ankylosing spondylitis (AS), psoriatic arthritis (PsA) and Crohn's disease (CD).

RA is thought to affect up to 1% of the population [1], and the prevalence for spondyloarthritides (SpA) (with their main subtypes, AS, undifferentiated SpA and PsA) is similar [2]. CD is less prevalent, but the total number of diagnosed cases in the US is estimated to be up to several hundred thousand patients, with 20 – 30% of cases occurring in people under 20 years of age [3].

Chronic inflammatory rheumatic diseases are considered highly debilitating, and a substantial proportion of work disability occurs among these disease groups. At least a third of the patients with RA, AS and PsA have severe disease [4]. Approximately 50% of RA patients discontinue work within 10 years of disease onset [5]. In a recent study [6], 23% of the patients in a cohort of 5054 patients had received Social Security disability payments, and 36% were work disabled due to RA symptoms. A threefold increase in unemployment has been reported in patients with AS. In a study conducted in Canada, patients with CD lost 26.1 days/year from work on average, compared with 6.3 days/year for the general population [7].

Conventional drug therapy for RA with non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids and disease-modifying antirheumatic drugs (DMARDs) are inadequate. In comparison to RA, the situation in patients with AS is more critical

because traditional DMARDs and systemic corticosteroids are less effective in this disease [8], which affects relatively young patients with a mean age of onset of 26 years. The course of AS is variable and the burden of disease is at least partly determined by the progressive stages [9]. The mortality of RA and AS patients has been reported to be increased, in severe AS as high as fourfold [10].

Current therapies for Crohn's disease, such as corticosteroids and azathioprine, induce remission in a significant percentage of patients [11]. However, some patients do not respond and relapses occur frequently, but not necessarily when treatment is completely discontinued. Corticosteroids work very well in the acute situation, but their prolonged use may lead to adverse side effects, such as infections, fractures relating to osteoporosis, Cushing's syndrome, undesired weight gain, skin changes and other problems.

In the past decade, anti-TNF-α therapy has emerged as an alternative to corticosteroids, azathioprine and other traditional DMARDs. Infliximab (Remicade®, Centocor, Inc., Malvern, PA, USA), the first mAb directed against TNF-α, was also the first of these mAbs clinically tested. The therapeutic potential of most of these mAbs has been substantiated over the past 5 years. Indeed, many clinical trials have demonstrated the effectiveness of mAbs in mediating inflammatory disease states, for approved indications such as RA and CD, and other upcoming indications such as AS and PsA. Infliximab, a chimaeric mAb, binds to TNF-α and effectively regulates and mediates the inflammatory processes involved in a number of other disease states as well. Clinical results using infliximab for various inflammatory and other diseases are reviewed in this article. The other approved anti-TNF drugs, etanercept and adalimumab, are not discussed in this review.

2. Mechanism of action and resistance

2.1 Role of TNF-α and mechanism of action of infliximab

Soluble TNF-α is a homotrimer of 17 kDa subunits, which is secreted mainly by macrophages and activated T cells. The mature 17 kDa polypeptide is derived by the proteolytic action of TNF-α-converting enzyme and released from the carboxyl-terminus of a 26 kDa transmembrane form of TNF-α which is oriented with its amino terminus inside the cell. Transmembrane TNF-α expressed on activated T cells has been reported to be part of the cell–cell contact-dependent signal that induces immunoglobin production by B cells. Transmembrane TNF-α expressed on cloned T cells infected with HIV-1 appears to be responsible for polyclonal B cell activation [12]. Another member of the TNF family, the CD40 ligand, is also expressed on activated T cells and has been shown to activate B cells efficiently [12,13].

Infliximab is a mAb that inhibits the biological activity of TNF. TNF-α has regulatory roles in a number of inflammatory diseases (e.g., RA and CD) [13]. TNF-α induces expression of chemokines such as growth-related peptide-α, macrophage inhibitory protein-2 (MIP-2), monocyte chemoattractant protein-1 (MCP-1), interleukin (IL)-8 and normal T cells. TNF-α stimulates the production of cytokines (e.g., IFN-γ, IL-1β and IL-6 and acute phase proteins). TNF-α induces the expression of endothelial adhesion molecules (e.g., E-selectin, intracellular adhesion molecule-1 [ICAM-1] and vascular cell adhesion molecule-1 [VCAM-1]).

Infliximab is a chimaeric mAb with murine variable regions and human IgG1 and κ constant regions. The size (149 kDa) and structure of infliximab are, therefore, similar to those of naturally-occurring antibodies. Infliximab binds to monomer subunits of human TNF-α. It is possible that by binding to TNF monomers, infliximab may slow or even prevent association of monomeric subunits of TNF forming bioactive trimeric TNF-α. Each infliximab molecule is capable of binding to two TNF molecules, and up to three infliximab molecules can bind to each TNF homotrimer, thereby blocking all receptor binding sites on TNF. Activation of the p55 and p75 TNF receptors initiates intracellular signal transduction cascades which lead to cell proliferation, upregulation of pro-inflammatory mediators or abnormal apoptosis [12,13].

Taken together, TNF-α is a molecule with regulatory and effector functions, which is important for the immune system. Infliximab is able to effectively block soluble and cell membrane bound TNF-α.

2.2 Possible explanations for resistance to infliximab treatment

Infliximab has been shown to be efficacious in the majority of patients. However, not all patients respond equally well. Many cases of treatment failure attributed to lack of infliximab efficacy may be at least in part explained by patient selection. Thus, patients who have disease states that are characterised by either low disease activity or advanced damage will respond marginally, or not at all, to anti-TNF therapy. The cellular and molecular mechanisms involved in TNF-α resistance to infliximab are speculative. Genetic differences in TNF promoters, TNF receptors, T cells, fibroblasts, antibodies and other cytokines such as interleukins IL-1, IL-6, IL-12, IL-15, IL-17 and IL-18, may be contributing factors.

There are only a few studies that address the question of predicting response to infliximab therapy. In an open-label trial, 24 patients treated with infliximab for CD, all of whom responded 1 week after treatment, relapsed over the next 16 weeks. Compared with patients who maintained remission, these patients who relapsed demonstrated an increase in both TNF-α secretion capacity and mucosal nuclear factor kappaB (NF-κB), which were detected before reactivation of clinical symptoms [14]. In another study on serological markers, anti-*Saccharomyces cerevisiae* antibodies (ASCA) and perinuclear antineutrophil cytoplasmic antibodies (pANCA) were found in 279 CD patients prior to anti-TNF therapy. However, no relationship between ASCA or pANCA and response

to therapy was observed. Lower response rates were observed for patients with refractory intestinal disease carrying the pANCA+/ASCA+ combination, although the difference was not significant ($p = 0.067$) [15]. Taken together, no firm conclusion can be drawn from these serologic data.

3. Pharmacokinetics

Both single-dose and repeated-dose pharmacokinetic (PK) characteristics of infliximab have been investigated [16]. Single intravenous infusions of infliximab, administered in doses ranging 1 – 20 mg/kg, in either CD or RA, show a predictable, well-defined, linear relationship between the dose administered and the maximum serum concentration (C_{max}). The clearance, volume of distribution (V_d) and terminal half-life ($t_{1/2}$) have been shown to be independent of infliximab dosage in patient subgroups based on age and weight. The median terminal $t_{1/2}$ of infliximab is in the range 8 – 10 days. The distribution of infliximab is primarily intravascular [17].

The PK profile of infliximab shows consistency across different demographic groups, as well as in paediatric versus adult patients and among patients with concurrent diseases of varying severity. However, differences in clearance between patients based on gender or marked renal or hepatic impairment have not been fully evaluated [17]. With repeated dose administration at 4- or 8-week intervals, following an initial 0, 2 and 6 week induction period, no accumulation of infliximab has been reported [18,19]. Twenty-five per cent of patients with RA who received 3 mg/kg every 8 weeks had undetectable serum infliximab concentrations at 8 weeks, 15% of those who received 3 mg/kg every 4 weeks had undetectable serum levels, and none of those who received 10 mg/kg at either 4- or 8-week intervals had undetectable serum levels. Among patients with CD who received either 5 or 10 mg/kg of infliximab, 20 and 12%, respectively, had undetectable serum infliximab concentrations at 8 weeks following infusion [20].

In combination therapy with methotrexate (MTX) (7.5 mg once-weekly), serum infliximab concentrations tended to be slightly higher when administered alone. While further studies are warranted, MTX may affect the rate of infliximab clearance by reducing its immunogenic potential [21]. The routes of metabolism and excretion of the drug are under evaluation at this time. More studies are warranted to evaluate the pharmacokinetics of infliximab in the elderly.

4. Efficacy and support studies

At present, infliximab is approved for use in conjunction with MTX to reduce the signs and symptoms, inhibit the progression of structural damage and improve physical function in patients with moderate-to-severe active RA who have had an inadequate response to MTX. The pivotal study for rheumatoid arthritis, titled 'An Anti-TNF Trial in Rheumatoid Arthritis with Concomitant Therapy', hereafter referred to as ATTRACT, was a randomised, double-blind, placebo-controlled clinical trial of anti-TNF chimaeric mAb (cA2) in patients with active RA despite MTX treatment. Infliximab is also approved for the reduction in signs and symptoms of CD, as well as for the induction and maintenance of clinical remission in patients with moderate-to-severe CD who have had an inadequate response to conventional therapy. The pivotal study for CD, titled 'A Crohn's Disease Clinical Trial Evaluating Infliximab in a New Long-Term Treatment Regimen', hereafter referred to as ACCENT I, was a randomised, double-blind, placebo-controlled trial of anti-TNF-α chimaeric mAb, infliximab, in the long-term treatment of patients with moderately-to-severely active CD. Randomised, placebo-controlled, double-blind trials have been supported by various prospective and retrospective open-label studies and case reviews, either corroborating the results of pivotal studies or evaluating efficacy in subpopulations.

Because infliximab has been approved for use in RA and CD, and the disease mechanisms involving TNF have been at least partly established, these disease states are not discussed in-depth. Instead, emphasis has been given to the clinical outcomes of TNF blockade and the use of infliximab in the treatment of other immune-mediated inflammatory diseases (i.e., AS, psoriasis, Behçet's disease and so on), discussed under section 5. These therapeutic areas require a more detailed mechanism of action to elucidate why infliximab was considered a possible treatment. An overview of the efficacy in these clinical trials is presented below.

4.1 Work in rheumatoid arthritis

4.1.1 Controlled studies

At least 12 controlled studies have demonstrated the efficacy of infliximab in RA. A total of > 1500 patients (infliximab-treated and placebo) have participated in US and European multi-centre trials since the first studies performed by Maini and colleagues in the early 1990s in the Kennedy Institute and at other European sites.

Inflixmab dosage has been in the range 1 – 20 mg/kg [18,22-24], although the most recent studies have demonstrated that a minimum dose of 3 mg/kg is necessary for clinical response [19]. Initially, one infusion was administered in order to study initial response and evaluate remission over time. However, induction regimens have been utilised since 1998, the most prominent being administered at weeks 0, 2 and 6, followed by administration every 8 weeks. The primary end point for efficacy in the pivotal studies was the American College of Rheumatology (ACR) response criteria (Table 1; all tables are in appendices pg 162 – 168).

At a minimum, 20% response was targeted (ACR20); although ACR50 and ACR70 have also been evaluated [16,19,25]. As part of the ACR indexed result, improvement in swollen and tender joints [22,26], reduction in the progression of joint damage [27,28] and the benefits of consistent treatment [29] were reported in almost all studies. Other areas of interest prompted by these studies have been reduction in the use of concomitant

medications [30], radiographic comparisons [31-36], pharmacokinetics [18,37], immunogenicity [23,18,24,38-40], time to onset of efficacy [30,41] and the safety of multiple infusions [18,39,42], as well as the rate of infusions [30,43] (Table 2).

One high profile outcome for this therapy is improvement of the quality of life, which is often linked to economic enhancement for the patient. Improvement in quality of life and function, as evaluated by the SF36 and Health Assessment Questionnaires (HAQs), may rise significantly and rapidly after treatment. Even partial resumption of daily physical functions, such as walking and dressing, may enable patients to be re-employed [44,45]. This potentially results in an economic benefit for the healthcare system. Indeed, patients with moderate-to-severe RA who had lower disease activity scores (DAS) as a result of infliximab treatment, accrued significantly lower overall medical costs in this analysis [44]. Increases in minimum clinically important differences in HAQs demonstrated improvement in functional status [45]. There are some indications that long-term follow-up of these trends continues. Presently, 3-year postmarketing data from the pivotal controlled study is still confirming success with infliximab treatment of RA [46].

4.1.2 Uncontrolled studies

Concurrent to controlled studies, prospective, open-label RA trials have demonstrated favourable results in juvenile RA [47-50], as well as successful tapering of concomitant therapies to evaluate infliximab monotherapy [51], efficacy and safety of infliximab and leflunomide [52], efficacy and benefits of infliximab and azathioprine [53], and efficacy and safety of infliximab among patients with inadequate response to etanercept [54] (Table 3).

Open-label immunogenicity studies have examined the behaviour of chemokines and the involvement of IL-8, MCP-1 [55], IL-1[α] and IL-1[β] [56]. A study with 263 patients at 50 sites in Germany confirmed the ACR20 results of the controlled trials [57]. Retrospective, open-label studies have suggested a neutral effect on pregnancy [58] and a favourable patient response to monotherapy, using HAQ evaluations [59]. Two retrospective studies in Belgium [53] and Canada [60] have confirmed the safety and efficacy reported in the controlled trials.

An infliximab safety registry compared 2896 patients prior to infliximab treatment with 230 patients already undergoing infliximab treatment for an average of 6 months. For the group already undergoing infliximab treatment, the mean number of tender joints was 42% less than the mean for the non-treated group; the mean number of swollen joints was 35% less than the mean for the non-treated group; global scores were 26% better than those for the non-treated group; pain scores were 26% better than those for the non-treated group; and the HAQ scores were 12% better than the non-treated group [61].

In a cohort of 296 patients with RA in Sweden, the mean number of hours worked per week at onset of therapy was 18.5 ± 1.3 (excluding pensioners and permanently disabled persons). After 2 years of anti-TNF-α therapy, the mean number of hours worked per week increased to 26.0 ± 2.5. There was no significant difference between the results for etanercept and infliximab in that study [62].

A few studies have been conducted on early RA, and preliminary outcomes have shown that infliximab is beneficial in mediating a prolonged therapeutic response, such as ACR50 [63,64] and radiologic progression [65]. Investigational juvenile RA trials have shown infliximab to be safe and effective for this as yet unapproved indication [48-50].

Several studies compared the efficacy of etanercept with infliximab. In one retrospective study, the median improvements in swollen joint count and in morning stiffness for patients administered with 3 mg/kg infliximab over 3 years were 90 and 82%, respectively, as compared with 75 and 67% for patients receiving etanercept during the same time period [66]. However, these indirect comparisons have clear limitations and no direct comparison has been performed so far.

These studies have provided clear evidence that infliximab works in RA. The drug is only approved in a dosage of 3 mg/kg, in combination with MTX. However, from the experiences in clinical practice it seems clear that some patients need higher doses and that not all patients tolerate MTX.

4.2 Work in Crohn's disease

4.2.1 Controlled studies

Six controlled studies have demonstrated the efficacy of infliximab in CD (Table 4).

A total of > 1100 patients (placebo and treatment) have participated in US and European multi-centre trials since 1995 [67-72]. In early trials, treatment ranged 1 – 20 mg/kg of infliximab [68,69], although more recent studies have demonstrated that a minimum dose of 3 – 5 mg/kg is necessary for response [71]. Often, only one infusion was administered to study initial response and monitor remission over time. However, induction regimens have been utilised since 1998, the most prominent being 5 mg/kg of infliximab administered at weeks 0, 2 and 6 followed by administration every 8 weeks. Primary efficacy end points in the controlled studies were a response represented by a change in the Crohn's disease activity index (CDAI), remission of disease activity at specified times and time to loss of response. Remission was defined as a CDAI score below 150 points [73]. In earlier studies, patients with active CD responded significantly to a single 5, 10 or 20 mg/kg infusion of infliximab treatment. CDAI scores in these earlier studies were markedly improved at 4 weeks [67,69], and later showed a significant response over placebo at 2 weeks [71]. Other end points in controlled studies were the effect of retreatment to sustain remission in patients who received a single infusion of 5, 10 or 20 mg of infliximab [72], the effect on endoscopic and histological healing [74] and the effect on downregulation of ileocolitis [75]. An induction regimen of 5 or 10 mg/kg of infliximab in a placebo-controlled trial was associated with a ≥ 50% reduction in the number of

draining fistulae over 3 months, and complete closure of fistulae was observed in 55% of patients who received 5 mg/kg of infliximab, compared with only 13% of patients who received placebo [69]. Of the 28 patients who had evaluable endoscopic results in the ACCENT I substudy, ~ 54% of patients who had received any dosage of infliximab as maintenance treatment had mucosal healing by week 54 [76]. Results of analysis of other secondary end points from the ACCENT I study (n = 573, 5 mg/kg of infliximab) demonstrated a preference for scheduled rather than episodic treatments [77], endoscopic healing [76,77], a preference for induction regimen over single dose [78], and grounds for reducing or discontinuing concomitant steroids [79].

As with RA, quality of life (QOL) improves significantly and rapidly with infliximab treatment [80,81], and patients have been able to return to the workplace [82-84]. As in RA, the economic impact to the health system has a diminishing potential [77,85]. Sustained remission and maintenance treatment reduce hospitalisations and the likelihood of surgery [86,87]. Recent studies have turned some attention to the determination of serum markers [15,88] and C-reactive protein (CRP) changes [89], and also to their role as early warnings of disease activity and their potential for serving as surrogate markers to monitor the disease cause and the effect of therapy (Table 5).

4.2.2 Uncontrolled studies

Prospective open-label studies in CD, using infliximab, corroborated the results of clinical trials [90-92]. Other studies have targeted specific areas of interest, such as efficacy of thalidomide for maintenance after induction of therapy with infliximab [93], and increased apoptosis of inflammatory cells causing enlarged lamina propria after treatment with infliximab [94] (Table 6).

Some retrospective, open-label studies in CD also corroborated the results of clinical trials [95-100]. Small case studies investigated unique applications of infliximab in inflammatory bowel diseases, such as remission of ileoanal pouch [101], healing of lesions in perineal cutaneous CD [102], effect on intestinal strictures [103,104], refractory pouchitis [105], extraintestinal manifestations [106], granuloma annulare [107], and CD in patients with HIV [108]. Paediatric CD applications of infliximab have also increased. Studies have been conducted to evaluate the pharmacokinetics of infliximab in children [109,110], steroid tapering in children [111], general improvement and remission in children [112-115], episodic treatment problems [116], in-office infusions [117] and QOL [118] (Table 7).

One study correlated the decrease in hospital resources as a direct result of use of infliximab in patients with CD. The records of 79 patients were analysed. For all patients, there was a 38% decrease in all surgeries ($p < 0.01$), an 18% decrease in gastrointestinal surgeries ($p < 0.05$), a 43% decrease in endoscopies ($p < 0.01$), a 66% decrease in emergency room visits ($p < 0.05$), a 16% decrease in all out-patient visits ($p < 0.05$), a 20% decrease in out-patient visits ($p < 0.01$), a 12% decrease in all radiological examinations ($p < 0.01$) and a 13% decrease in non-plain films ($p < 0.01$). For patients with fistulising CD ($n = 37$), there was a 59% decrease in hospitalisations ($p < 0.05$), a 66% decrease in all surgeries ($p < 0.01$), a 59% decrease in gastrointestinal (GI) surgeries ($p < 0.01$), a 27%, 26% and 70% decrease in all-cause, GI and surgical out-patient visits, respectively ($p < 0.05$ for all), a 64% decrease in emergency room visits ($p < 0.05$), a 40% decrease in all radiological examinations ($p < 0.05$), and a 61% decrease in non-plain films ($p < 0.050$). Patients with luminal disease ($n = 42$) had a 52% decrease in the number of endoscopies performed ($p < 0.05$) and a 69% decrease in emergency room visits ($p < 0.050$) [119].

In summary, infliximab works in CD. Permanent dosing seems to have advantages over single shot approaches, but the optimal duration of therapy and the advantages over conventional approaches with steroids and azathioprine need more substantiation.

5. Expanding therapeutic indications

5.1 Preliminary work in ankylosing spondylitis

The spondyloarthritides are a group of chronic inflammatory diseases, of which AS is thought to be the most common [2,120]. Characteristics of the disease are the involvement of the axial skeleton starting in the sacroiliac joints [121] and the entheses [122], as well as the synovium. The disease has a strong genetic association [123]. About 90% of Caucasians with AS have the human leukocyte antigen (HLA)-B27 gene, an allele of the major histocompatibility complex [124,125]. The diagnosis is based on the modified New York criteria for AS [126]. The major manifestations of the disease are inflammatory low back pain, which is a consequence of sacroiliitis, spondylitis and, possibly, enthesitis of axial structures. A significant percentage of the patients also have peripheral arthritis and anterior uveitis. Other organ involvement may include heart disease (especially of the aortic root), kidney involvement by amyloidosis, and lung disease [127]. The mortality of patients with clinically relevant AS is increased by a factor of ~ 1.5 [8].

Abundant TNF-α mRNA has been found in sacroiliac joints biopsies from patients with AS [128], suggesting a pathogenic role for TNF. NSAIDs are useful for management of pain in some patients but are ineffective as disease modifying agents.

Assessment of AS has been defined by a core set published by the Assessments in Ankylosing Spondylitis (ASAS) group [129]. This study group has recently proposed improvement criteria on the basis of NSAID trials [130]. The most frequently used tools for clinical measurements in AS nowadays are the Bath indices: BASDAI (Bath Ankylosing Spondylitis Disease Activity Index) [131], BASFI (Bath Ankylosing Spondylitis Functional Index) [132], BASMI (Bath Ankylosing Spondylitis Metrology Index) [133] and BASRI (Bath Ankylosing Spondylitis Radiology Index) [134], all using a 10 cm visual analog scale. Other indicators are the erythrocyte sedimentation rate (ESR), CRP, pain levels and physician/patient HAQs.

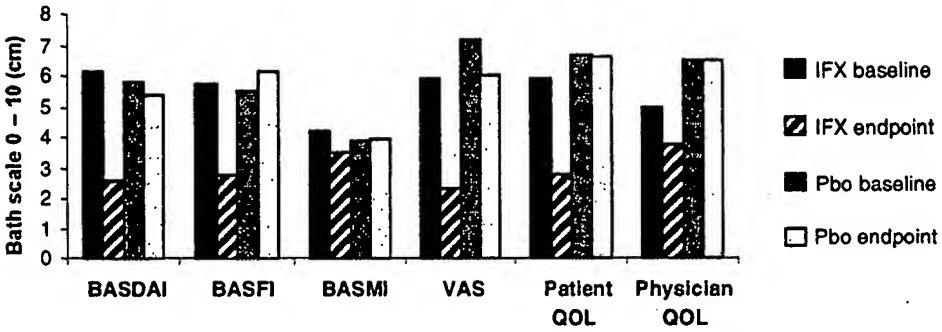

Figure 1. Ankylosing spondylitis clinical experience. IFX (n = 263); Pbo (n = 55).
AS: Ankylosing spondylitis; BASDAI: Bath AS disease activity index; BASFI: Bath AS functional index; BASMI: Bath AS metrology index; IFX: Infliximab; Pbo: Placebo; QOL: Quality of life; VAS: Visual analog scale.

Two pilot studies were performed in Berlin [135] and Gent [136], which suggested definite clinical benefits of infliximab in AS and PsA. Both groups also performed randomised, double-blind, placebo-controlled clinical trials [137,138] (Table 8). The Berlin group conducted a placebo-controlled multi-centre study of 70 patients, 35 of whom received 5 mg/kg infliximab at weeks 0, 2 and 6 weeks, and 35 of whom received placebo; an evaluation was performed at 12 weeks. Eighteen of thirty-five infliximab-treated patients (53%) had ≥ 50% reduction in disease activity (BASDAI), as compared with three of thirty-five placebo-treated patients (9%). At 12 weeks, placebo-treated patients received crossover treatments with infliximab, starting with an induction dose of 5 mg/kg at weeks 12, 14 and 18, followed by an infusion every 6 weeks. At week 18, the placebo-crossover patients experienced an equivalent reduction in disease activity as compared with patients who initially received infliximab and completed their induction therapy at weeks 0, 2 and 6. At week 54, patients initially assigned to infliximab and the placebo-crossover patients responded equivalently, with 53.3 and 51.4% of patients, respectively, achieving at least a 50% reduction in disease activity [139]. There was clear improvement of inflammatory back pain, peripheral arthritis and enthesitis. Importantly, function, mobility and QOL also improved significantly. After 1 year, 78% of the patients are still receiving infliximab therapy, and continuous improvement was reported over 52 weeks, without any evidence of diminishing efficacy.

There is preliminary evidence that active and chronic changes of AS, as detected by a novel magnetic resonance imaging (MRI) scoring system, improved after 3 months of treatment [140,141].

Van den Bosch also conducted a placebo-controlled, multicentre, randomised study, in which 20 patients received placebo and 20 patients received 5 mg/kg of infliximab at weeks 0, 2 and 6, with a follow-up evaluation in week 12. Mean BASDAI scores dropped from 5.9 to 2.7 cm in the infliximab group and from 5.3 to only 5.0 cm in the placebo group. Mean BASFI scores decreased from 4.7 to 2.7 cm in the infliximab group but increased from 5.9 to 7.2 cm in the placebo group. There were also significant reductions in morning stiffness, peripheral joint pain, tender joint count and patient and physician global assessments for patients who received infliximab, whereas these factors increased for patients who received placebo. ESR, CRP and swollen joint counts also decreased in infliximab-treated patients. An extension of this trial demonstrated the safety and efficacy of a retreatment regimen of 10 mg/kg administered every 14 weeks to patients previously given 5 mg/kg at weeks 0, 2 and 6, in order to maintain the initial improvement. Every retreatment sustained a significant improvement ($p < 0.01$, compared to baseline) [142] (See Table 2).

There have been nine open-label prospective trials conducted in 197 patients, with infliximab dosages of 3 – 5 mg/kg at weeks 0, 2 and 6, with follow-up evaluations at week 12 or 14 [135,136,145-149] (Table 8).

Including the 55 patients treated with infliximab from the controlled studies, Figure 1 presents the clinical trial experience results and Figure 2 presents the laboratory data results for a total of 252 patients with AS. There have also been several case studies which demonstrated comparable levels of improvement [150-153].

Additional studies have analysed T cells, cytokines [154], polymorphisms [155], interleukins, V-CAM1, MCP-1, MMP-3, MMP-11 [156], protein amyloids [157] and vascularity [158], in order to further examine the mechanism of action for anti-TNF-α therapy in the spondylarthritides. It was shown that the nonspecific and the antigen-specific cytokine secretion was definitely influenced by infliximab therapy.

There are clear indications that etanercept is also effective in AS [159-162]. In a 6-month double-blind, placebo-controlled, Phase III clinical trial in 30 patients, etanercept was efficacious in treating AS (57% of patients demonstrated 50% ACR improvement in 6 weeks) [162]. The onset of action might be somewhat slower with etanercept, but overall no clear differences between infliximab and etanercept have been established.

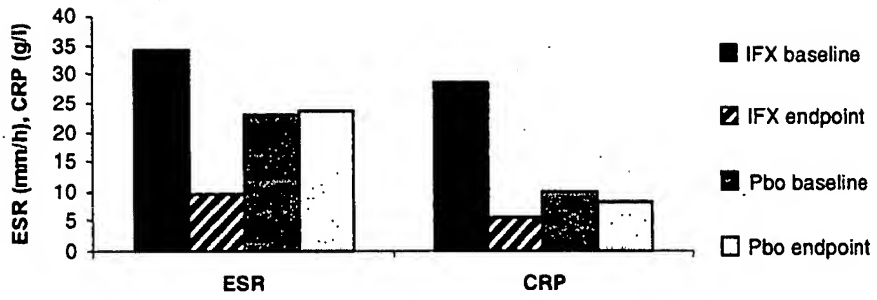

Figure 2. Ankylosing spondylitis laboratory experience. IFX (n = 263), Pbo (n = 55).
CRP: C-reactive protein; ESR: Erythrocyte sedimentation rate; IFX: Infliximab; Pbo: Placebo.

Overall, studies in AS have shown that anti-TNF therapy is effective in AS and represents a major breakthrough in the therapy of this disease, especially because, unlike for RA, there are almost no alternative therapies.

5.2 Preliminary work in Behçet's disease

Behçet's disease is an idiopathic, chronic, relapsing, immune-mediated, inflammatory disorder, which is characterised by painful mouth sores, skin blisters, genital sores and swollen joints. A major threat to patients is blindness resulting from uncontrolled panuveitis. Serum levels of TNF and soluble TNF receptors were shown to be elevated in patients with active disease. There are seven case studies of infliximab use in Behçet's disease. All patients received dosages of infliximab in the range 2 – 10 mg/kg as episodic treatments or as a regimen at weeks 0, 2, 6 and every 8 weeks thereafter. The clinical manifestations of disease in seven patients at baseline consisted of oral, anal and genital ulcerations, which improved in a few weeks and cleared later in the study [163-168]. All patients were taking concomitant medications, some of which were discontinued as a result of treatment. Indications suggest that infliximab is a promising drug for severe Behçet's disease (see also section 5.6.).

5.3 Preliminary work in psoriasis/psoriatic arthritis

It is believed that psoriasis is driven by activated memory T cells, namely CD4+ (dermal) and CD8+ (epidermal). Anti-TNF-α binds to secreted cytokines and suppresses their activity in the development of psoriasis and other inflammatory skin diseases. It also appears to inhibit migration of T cells and downregulates both T cell activation and activity [169].

Psoriatic disease activity is measured by the Psoriasis Area and Severity Index (PASI) score. For psoriatic arthritis, the Psoriatic Arthritis Response Criteria (PsARC) score is now commonly used [170].

In a double-blind, randomised, placebo-controlled infliximab study in patients with moderate-to-severe plaque psoriasis (n = 33), only 18% of patients who received placebo achieved the primary end point of good, excellent or clear ratings on the physician's global assessment, as compared with 82% of patients who received 5 mg/kg of infliximab and 91% for those given 10 mg/kg of infliximab. A 75% improvement in PASI scores was the secondary end point. This outcome was achieved by 18% of placebo-treated patients, 82% of 5 mg/kg infliximab-treated patients and 73% of 10 mg/kg infliximab-treated patients. The median time to response was 4 weeks [171]. In a subset analysis of this study, infliximab monotherapy normalised keratinocyte differentiation and decreased inflammation in skin biopsies from these 33 patients, indicating further that TNF-α may be pivotal in the pathogenesis of psoriasis [172]. In an unblinded study extension, 48% of patients maintained a 75% improvement in PASI scores through 26 weeks [173].

A double-blind, randomised, placebo-controlled infliximab study in patients with PsA (n = 102) was recently completed [174]. Placebo and infliximab 5 mg/kg were administered at weeks 0, 2, 6 and 14, followed by open-label 5 mg/kg infliximab treatment and follow-up through week 50. In the infliximab group, 70.6% (36 of 51) patients achieved 20% improvement in American College of Rheumatology (ACR) score (ACR20) and 52.9% (27 of 51) achieved ACR50. In the placebo group, 9.8% (5 of 51) achieved ACR20 response criteria. There were no significant infusion reactions, no opportunistic infections and no increase in systemic infections. However, there was one case of joint infection. Similar positive effects have been reported with etanercept [175]. Anti-TNF therapy is clearly beneficial for joint and skin involvement in psoriasis and PsA.

Five prospective open-label PsA studies [176-181] have demonstrated marked improvement in disease activity in patients treated with 3 – 5 mg/kg of infliximab after an induction regimen at weeks 0, 2 and 6. Ogilvie *et al.* [176], Schopf *et al.* [177] and Cherouvim *et al.* [178] demonstrated a decrease in the range of pretreatment PASI scores of 2.1 – 31.7 to a post-treatment range of 0.2 – 11.2. Feletar *et al.* demonstrated that infliximab had a moderate response for joint disease that was not well sustained, but demonstrated a marked improvement in psoriasis, which was well maintained [179].

Psoriasis-associated pruritus scores dropped from 2.5 ± 0.26 at week 0, to 0.8 ± 0.3 at week 14 (scale of 0 – 3) in the Schopf study [177], in which patients were treated with 5 mg/kg infliximab at weeks 0, 2 and 6. Acanthosis (epidermal thickness) decreased from 0.41 ± 0.06 mm to 0.14 ± 0.02 mm in 10 weeks. In a study conducted by Cherouvin *et al.*, morning stiffness decreased from a mean of 95 min to 27 min. The mean number of tender joints decreased from 22.2 to 7.2, and swollen joints from 9.6 to 2.1 [178] (Table 9).

Four retrospective, open-label psoriasis and PsA studies [180-183] also demonstrated marked improvement in disease activity. Stevens *et al.* [181] and Fleischmann *et al.* [182] reported a decrease in the number of swollen joints from a pretreatment range of 7.7 – 15.5 to a post-treatment range of 1.9 – 3.1 when infliximab was administered for an average dose of 3.6 – 3. 9mg/kg during an average of 4 – 5 infusions. Four case studies in psoriasis and PsA [183-186] demonstrated dramatic improvement in disease status within 1 – 7 days, and four additional case studies [187-190] demonstrated dramatic improvement in disease status after multiple infusions. In some instances concomitant medications were discontinued. As a result of the success of infliximab in the treatment of psoriasis and PsA in these studies, full clinical trials are in progress.

5.4 Preliminary work in other dermatological diseases

Retrospective data were collected for 57 patients who were treated with infliximab for various skin disorders. They were given episodic infusions of 5 mg/kg infliximab, sometimes repeated monthly. The following indicates patients with marked improvement in each dermatologic disease: 19 patients with psoriasis (33.3%); 10 patients with hidradenitis suppurative (17.6%); 2 patients with marked and 6 patients with moderate pyoderma gangrenosum (PG) (12.3%); and 9 patients with pityriasis rubra pilaris (15.8%). Improvement was also demonstrated in 2 patients with eosinophilic fasciitis, 2 patients with panniculitus, 1 patient with Stevens–Johnson syndrome, 2 patients with acne fulminans, 2 patients with scleroderma and 1 patient with necrobiosis lipoidica diabeticorum. Conditions worsened after infliximab treatment in 2 patients with discoid lupus, 1 patient with foreign body granuloma and 2 patients with atopic dermatitis [183].

5.5 Preliminary work in pyoderma gangrenosum

PG is an idiopathic and chronic ulcerating skin disease, frequently associated with inflammatory bowel disease. It occurs predominantly in the lower extremities and peristomal areas. Treatment presently consists of local wound treatment, corticosteroids, immunomodulators, antibiotics, 5-aminosalicylate compounds and surgery [191,192]. PG responds to cyclosporin, indicating that T lymphocytes may be involved in pathogenesis [193], and there is evidence of neutrophilic infiltration of the dermis and lymphocytic infiltrations of the blood vessels [191].

Infliximab treatment in PG has been documented in only a few case studies [187,191,192,194-197]. No double-blind, randomised, placebo-controlled studies have been published, to date. In these case studies, all patients received 4 – 10 mg/kg infusions of infliximab. Baseline conditions consisted of skin lesions, fistulae, pustulae, ulcerations, diarrhoea, colonic perforations, pouchitis, Sweet's syndrome and pain. Steroid tapering was conducted in a few cases. Outcomes consisted of symptom relief as early as 24 h, or within a few days following infliximab therapy. Lesions and fistulas closed and CRP protein levels decreased. In some instances there was complete healing within a few weeks following treatment.

5.6 Preliminary work in uveitis

Uveitis is an inflammatory process of the uveal tract structures of the eye, commonly caused by infections, trauma, lymphoproliferative disorders and ischaemia, but in some instances, the cause has an autoimmune component [194]. For this reason, uveitis is a common manifestation in Behçet's disease, Crohn's disease, juvenile RA, spondyloarthritis and other inflammatory immune disorders [198]. Considering the success of anti-TNF therapy in the aforementioned disease states, infliximab was evaluated in several uveitis case studies.

A single patient with spondyloarthritis presented with anterior uveitis, resulting in vitritis and chronic anterior and intermediate uveitis, with a positive Tyndall phenomenon ++ (defined as the occurrence of visible floating particles in gases and liquids that are illuminated by a ray of sunlight and are viewed at right angles to the illuminating ray). After 1 week, there was a marked improvement in the Tyndall phenomenon. Improvement was sustained through 14 weeks until a relapse necessitated a second infusion. The patient's condition remained stable for 58 weeks (n = 1) [199].

One 5 mg/kg intravenous dose of infliximab was administered to five patients with Behçet's disease at the time of immediate onset of their last relapse of panuveitis. Remission of ocular inflammation was evident after 24 h, with complete suppression after 7 days in all patients. Follow-up evaluation at day 14 showed improvement in retinal lesions, visual acuity, anterior chamber cell involvement, vitreous haze and vasculitis among all patients. There were no observed side effects [200].

In a study, seven patients with HLA-B27+ anterior uveitis were given a single infusion of 10 mg/kg infliximab as monotherapy. There was improvement in clinical symptoms and a decrease of anterior chamber cells within 7 days for three patients, after 11 days for one patient, after 17 days for one patient and after 39 days for another patient. One patient could not achieve complete resolution with infliximab therapy. However, after 120 days, three of the seven patients relapsed and were given steroids [201]. Three patients suffering from a non-responsive chronic uveitis showed no remission when treated with systemic or local corticosteroids combined with MTX. Two of the three patients received three doses of 5 mg/kg of infliximab at 0, 2 and 6 weeks. The third patient received only one dose of 5 mg/kg of infliximab. All three

patients achieved remission within 2 weeks. Visual acuity improved in all patients, and treatment with corticosteroids was discontinued in two patients and tapered in the third patient [201].

In a similar study, three patients with associated HLA-B27 anterior uveitis demonstrated different responses. The first patient, who received 10 mg/kg of infliximab, relapsed 5 months after the last infusion. The second patient, however, who received 5 mg/kg of infliximab, remained in remission 5 months after the last infusion. Furthermore, the third patient, who received 5 mg/kg of infliximab, remained in remission 3 months after the last infusion. The results from individual patients show the need for individual assessment and dose tailoring [202].

Two other case studies have reported positive results with an induction regimen consisting of 3 and 5 mg/kg of infliximab, with ocular aching, clouding and photophobia resolving in days [203,204]. A study in eight paediatric patients with refractory chronic uveitis in juvenile idiopathic arthritis demonstrated a decrease in cellularity in five of eight patients at 3 months, but at 6 months, two patients experienced flare of uveitis [205]. Seven patients with refractory posterior uveitis were treated with infliximab in an open-label study. No adverse events and no ocular or systemic exacerbation were observed, and visual acuity improved or became stabilised in every patient [206].

A 5 mg/kg single infusion of infliximab was administered to 14 patients with Behçet's disease-associated, sight-threatening panuveitis. One day following treatment, there was a significant improvement in visual acuity, a decrease in anterior chamber cells and a decrease of vitreous haze. Acute ocular inflammation and macular oedema resolved [207]. Four patients with resistant uveitis, who were previously unresponsive to corticosteroids or developed unacceptable toxicities, responded well to 3 – 5 mg/kg of infliximab, with no observable toxicities [208].

There are no randomised studies on the efficacy of anti-TNF therapy in uveitis. The case studies cited above give the indication that infliximab may be beneficial for some but not all patients [209,210].

5.7 Preliminary work in ulcerative colitis

A double-blind, placebo-controlled study, which originally had 60 patients enrolled, was terminated due to slow enrolment and was recharacterised as a prospective study of 11 patients treated for ulcerative colitis (UC), 8 of whom received a single infusion of 5, 10 or 20 mg/kg of infliximab and 3 of whom received placebo. The primary end point was treatment failure within 2 weeks, defined as one of the following:

- failure to achieve a clinical response by virtue of requiring treatment with corticosteroids given at a dosage of at least 60 mg/day
- disease requiring treatment with cyclosporin therapy or other immunomodulators
- the need for a colectomy
- death

A clinical response was defined as a score of < 10 and a 5 point reduction relative to baseline (modified Truelove and Witts score). At 2 weeks, four of the eight patients treated with infliximab achieved clinical response, compared with none of the three patients treated with placebo [211]. Although this study is too small to draw any major conclusions, the data initiated further research on the efficacy of infliximab in UC.

In another study, 17 steroid-naive patients, whose UC was refractory to mesalamine, were administered an induction regimen of 5 mg/kg of infliximab at 0, 2 and 6 weeks. Patients who achieved remission were then treated every 2 months, while patients who failed to respond began treatment with prednisone. Testing was conducted using the pANCA immunofluorescence assay associated with 80% of UC cases, and the ASCA assay associated with 80% of CD. The results showed that CRP concentrations decreased from baseline between week 12 and week 24 in all subgroups, differentiated on the basis of pANCA-or ASCA-positivity. Thus, treatment with infliximab resulted in improvement of colitis for each of these subgroups, possibly suggesting that infliximab is effective across the spectrum of inflammatory bowel diseases [212].

In a prospective study, 32 patients with UC that was refractory to aminosalicylates and/or 6-mercaptopurine, refractory to corticosteroids or requiring corticosteroid treatment, were administered 5 mg/kg of infliximab. The mean Ulcerative Colitis Clinical Activity Index (UCCAI) score decreased from 15.8 ± 0.6 to 3.2 ± 0.8 ($p < 0.001$). Infliximab was found effective in this study. Clinical improvement was accompanied by significant endoscopic and histologic improvement of the colon mucosa [213].

In another study [214], a total of 27 patients with active UC received in-patient (37%) and out-patient (63%) infliximab as single (52%) or multiple (2 – 15) infusions (48%). Twelve patients (44%) achieved remission and six patients (22%) had partial response. Nine patients had no response; five subsequently underwent total colectomy. The median time to achieve response and remission was 4 days, and the median duration 8 weeks. Nine of the eighteen patients who responded experienced 19 relapses; 18 of these relapses (95%) were successfully treated with repeat infusions. Steroid-refractory patients were less likely to respond to infliximab therapy than steroid-responsive patients (33 versus 83%; $p = 0.026$). No other factors were predictive of response to infliximab. Two patients developed serious adverse events, including death in one case. The authors concluded that there is preliminary evidence suggesting effectiveness of infliximab in the treatment of UC, including medically refractory severe disease. Individuals who are refractory to corticosteroids, however, may be unlikely to respond to infliximab. A randomised, controlled trial is needed to further investigate the efficacy of infliximab in patients with UC.

5.8 Preliminary work in other diseases

There have been off-label uses of infliximab in diseases known to have an underlying immune-inflammatory mechanism, but not necessarily involving TNF-α. Case reports have been

published demonstrating moderate-to-marked to dramatic improvement (Table 10).

The anticytokine medication programs initiated for chronic heart failure (CHF) were halted because of limited efficacy and side effects in the high dose group. The efficacy of anti-TNF therapy in low dosages for patients with CHF may need to be determined in future studies [215]. The safety issues are discussed under section 6.6.

6. Safety and tolerability

Infliximab inhibits cytokine production and/or action, primarily by binding to TNF-α in its free state as well as bound states. The most common reason for discontinuation of treatment is infusion-related reactions (dyspnea, flushing, headache and rash). Adverse events have been reported in a higher proportion of patients with RA who received 10 mg/kg of infliximab than in those who received 3 mg/kg of infliximab; however, no differences were observed in the frequency of adverse events between the 5 mg/kg dose and 10 mg/kg dose in patients with CD [17].

In a recent paper on therapy of AS, the issue of safety has been discussed quite extensively [8].

6.1 Infusion-related reactions

An infusion reaction, defined as any adverse event occurring during an infusion or within 1 – 2 h after an infusion, occurred in 22% of infliximab-treated patients in all clinical studies, compared with 9% of placebo-treated patients. Infusion reactions have occasionally been accompanied by nonspecific symptoms such as fever or chills, or by cardiopulmonary reactions (primarily chest pain, hypotension, hypertension or dyspnea) and < 1% are accompanied by pruritus, urticaria or the combined symptoms of pruritus/urticaria and cardiopulmonary reactions. Serious infusion reactions occurred in < 1% of patients and included anaphylaxis, convulsions, erythematous rash and hypotension. Patients who developed antibodies to infliximab have been shown to be ~ 2 – 3 times more likely to have an infusion reaction than those who did not. Use of concomitant immunosuppressant agents such as MTX appears to reduce the risk for antibodies to infliximab and infusion reactions. However, whether this justifies using infliximab only in combination with MTX is still a matter of debate. In postmarketing experience, rare cases of anaphylactic-like reactions, including laryngeal/pharyngeal oedema and severe bronchospasm and seizure have been associated with infliximab administration. However, infusion-related side effects can, in general, be easily handled in experienced centres.

6.2 Infections

In clinical studies, infections requiring treatment were reported in 36% of infliximab-treated patients (average of 56 weeks of follow-up) and in 26% of placebo-treated patients (average of 41 weeks of follow-up). With longer observation periods (i.e., 6 months following the last patient infusion), the rate of infection was similar for both groups. The infections most frequently reported were respiratory tract infections (including sinusitis, pharyngitis and bronchitis) and urinary tract infections. No increased risk of serious infections or sepsis was observed with infliximab compared with placebo in the clinical studies in which all patients received MTX concomitantly. Among infliximab-treated patients, serious infections included pneumonia, cellulitis, abscess, skin ulceration, sepsis and bacterial infection. In the ATTRACT study, one patient died with miliary tuberculosis and one died with disseminated coccidioidomycosis. In the ACCENT study, one patient was diagnosed with tuberculosis. In the Berlin AS trial one patient developed systemic tuberculosis. Other cases of tuberculosis, including disseminated tuberculosis, have also been reported in postmarketing experience. Most of the cases of tuberculosis occurred within the first 3 months after initiation of therapy with infliximab and may reflect recrudescence of latent disease. In postmarketing experience, infections have been observed with various pathogens including viral, bacterial, fungal and protozoal organisms. Infections have been noted in all organ systems and have been reported in patients receiving infliximab alone or in combination with immunosuppressive agents. Concern about tuberculosis infection in patients treated with anti-TNF agents has led to a labelling change, recommending that all patients to be treated with these drugs undergo screening for tuberculosis prior to treatment. Patients with evidence of tuberculosis infection should be treated for the infection prior to the initiation of anti-TNF therapy, or the drug should not be used in these cases [216]. Consequent screening by purified protein derivative (PPD) testing and X-ray before the start of treatment has already decreased the incidence of tuberculosis on infliximab therapy.

6.3 Autoantibodies/lupus-like syndrome

In the ATTRACT study, through week 102, 62% of infliximab-treated patients developed antinuclear antibodies (ANA) between screening and the last evaluation, compared with 27% of placebo-treated patients. Anti-dsDNA antibodies developed in ~ 15% of infliximab-treated patients, compared with none of the placebo-treated patients. No patients had CNS or renal involvement. No cases of lupus-like reactions have been observed in up to 3 years of long-term follow-up.

In the Berlin AS trial, two ANA+ DNA- cases with transient polyarthritis have been observed.

In the ACCENT I study, 56% of infliximab-treated patients developed ANA between screening and the last evaluation, compared with 35% of placebo-treated patients. Anti-dsDNA antibodies developed in ~ 34% of infliximab-treated patients, compared with 11% of the placebo-treated patients. No patients had CNS or renal involvement. There were two patients treated with infliximab maintenance who developed lupus-like symptoms. One was attributed to the antihistone antibody and the other was attributed to ANA and anti-dsDNA. Lupus-like

syndrome and multiple sclerosis-like syndromes have, however, been rarely reported in the postmarketing experience.

6.4 Malignancies/lymphoproliferative disease

In completed clinical studies of infliximab for up to 102 weeks, 18 of 1372 (1.3%) patients developed 19 new or recurrent malignancies of various types, over 1430 patient-years of follow-up. These were non-Hodgkin's B cell lymphoma, breast cancer, melanoma, squamous, rectal adenocarcinoma and basal cell carcinoma. There are insufficient data to determine whether infliximab contributed to the development of these malignancies. The observed rates and incidences were similar to those expected for the populations studied.

Eight cases of lymphoma were reported in postmarketing experience with infliximab. This is tantamount to a rate of 6.6 cases/100,000 persons, from drug approval to March 2001. This infliximab rate was below the age-adjusted incidence of lymphoma in the US from 1992 – 1998, which was 18.3/100,000. Due to the limitations of the Med-Watch system, the number of cases are believed to be understated. Other immunosuppressive agents were used by these patients at the time of the study. These cases do not establish a cause–effect relationship. However, they do underscore the need for caution in clinical use [216].

Taken together, there is no concern regarding the issue of malignancy as it stands now. However, further collection of data in large databases is needed to detect long-term side effects.

6.5 Immunogenicity

Treatment with infliximab can be associated with the development of antichimaeric antibodies to infliximab. Approximately 10% of patients have been shown to be antibody positive. The majority of antibody-positive patients had low titres. Patients who were antibody-positive were more likely to experience an infusion reaction, but this correlation was not very strong. Autoantibodies were primarily induced when infliximab was given in a low dosage of 1 mg/kg. The presence of antibodies to infliximab in the serum is known to interfere with the interpretation of the analyses for antibodies to infliximab.

6.6 Congestive heart failure

In a Phase II study evaluating infliximab in New York Heart Association (NYHA) Class III/IV CHF patients (left ventricular ejection fraction ≤ 35%), higher incidences of mortality and hospitalisation resulting from worsening heart failure were observed in infliximab-treated patients, especially those treated with the 10 mg/kg dose.

As it stands now, infliximab should definitely not be given to patients with clinically significant heart failure (NYHA III and IV).

6.7 Long-term safety follow-up

A follow-up programme to the pivotal trials collected information on new autoimmune diseases, malignancy and death over 3 years following the last study infusion, and information on serious infections at 6 months following the last infusion. No excess of serious infections, malignancies or deaths were observed with infliximab in these pivotal trials. There were two cases of tuberculosis and two cases of opportunistic infections reported. Five of 1372 patients (0.4%) developed clinical signs of lupus-like syndrome. All patients had resolution of these symptoms and no patients had renal or CNS involvement. The incidences of major safety events during long-term follow-up in infliximab patients were comparable to those observed in placebo-treated patients. The number of non-lymphoma cancers reported in clinical trials was in agreement with those expected for the normal population, according to the Surveillance, Epidemiology and End Results Database (SEER). The long-term safety of infliximab remains consistent with earlier observations [46].

Beginning in January 2001, > 5000 patients were enrolled in a long-term safety registry. Of the first 3188 patients enrolled, 1-year validated results reported no obvious increases in adverse events, compared to what might be expected in a similar RA population [217].

7. Expert opinion

MAbs have introduced a new era in the treatment of some immune-mediated inflammatory diseases such as RA, AS and CD. Modern biologic anti-TNF-α therapies are cutting across traditional boundaries of clinical practice. These illnesses seem to share a common inflammatory pathway in which TNF-α appears to play an important role. The results of clinical trials with several agents are promising, and the advances achieved by anti-TNF-α treatment of various inflammatory conditions associated with rheumatic symptoms seem to emerge increasingly clear.

Infliximab, the first of these mAbs to be clinically tested, and the subject of this review, was first approved for CD in 1998 and for RA in 1999. Clinical development programs for AS and PsA are underway. Preliminary results of studies of infliximab in the treatment of SpA have demonstrated benefit for both peripheral and axial inflammation and arthritis. Infliximab appears effective for other extra-articular features or inflammatory manifestations of immune-mediated diseases, such as enthesitis, psoriasis, colitis and uveitis. In RA, progression of structural damage is inhibited by treatment with infliximab. This has not been properly studied in AS or PsA, but trials are underway.

Based on its therapeutic success in CD, infliximab is being assessed in other inflammatory conditions of the GI tract. Although there are some promising preliminary data [211-213], more work is needed to document the efficacy of infliximab in UC.

According to case reports, there are further indications that infliximab may be beneficial in other immune-mediated inflammatory diseases which involve TNF-α. There have been some open-label studies on Behçet's disease, suggesting

that refractory cases associated with uveitis, in particular, can also be treated with infliximab.

The positive response to anti-TNF therapy in many of these inflammatory diseases appears to exceed the response reported with conventional therapies, including DMARDs. In clinical trials, DMARD refractory patients with RA, CD, AS and PsA have been shown to benefit significantly from infliximab therapy. Other anti-TNF-α therapies have had similar success in the treatment of RA, PsA and AS, but not CD, suggesting unique differences in the various anti-TNF agents.

As reported, the onset of action of infliximab appears to be rapid in certain diseases, and patients continue to improve during therapy, often with improvement of symptoms over ≥ 2 years. Infliximab has been approved for episodic and maintenance therapy of CD, in the US. Maintenance treatment, to date, has been the standard approach in the inflammatory arthritides. Although antibodies to infliximab may arise more frequently with episodic therapy, no apparent loss of efficacy has been observed in CD trials.

There is clinical trial experience with infliximab monotherapy in CD, as well as anecdotal and controlled experience with infliximab monotherapy in AS and psoriasis. Whether monotherapy with infliximab is as efficacious and as safe as the combination with MTX in RA or in these diseases is yet to be defined.

A number of investigators have reported that concomitant medication with glucocorticoids or NSAIDs can be tapered or discontinued during infliximab therapy [30,51,79,111]. This is likely to affect the development of osteoporosis in these patients who are at increased risk of fracture.

The optimal dosage of infliximab is 3 – 5 mg/kg. Higher doses seem to provide no further benefit. Individual dosing in this range, although not systematically studied, is often performed in clinical practice for pragmatic reasons.

The length of time for which infliximab can be administered has not been determined. There are patients who have already received the drug for 3 or 4 years with ongoing benefit and no serious side effects; however, more data from clinical experience is necessary to establish the long-term safety and efficacy of infliximab.

Some patients in clinical trials have had a limited clinical response to infliximab. The reasons for this are unclear. However, depending on the type disease, certain parameters such as low initial disease activity, low CRP or accumulated damage prior to therapy, may account for this apparent lack of response.

Theoretically, some patients may not respond because a different cytokine may be their primary mediator of inflammation. However, this has not yet been proven. In clinical trials, patients may not demonstrate significant improvement based on the primary end point measure and, thus, may be classified as 'non-responders'. As reported in the ATTRACT cohort, these same patients may simultaneously demonstrate improvement in QOL or radiographic joint damage improvement. There are no data suggesting that a positive response to infliximab is largely influenced by age, gender or race.

QOL is an increasingly important issue in healthcare, especially in disease, which is not immediately life-threatening. Subanalyses of clinical trial data have indicated that slowing disease progression through treatment with infliximab may have an impact on disability and, therefore, employability. Other benefits of treatment may include participation in sports and the resumption of a relatively normal lifestyle, unrealised prior to treatment. Especially in AS, but also in RA and PsA, there has been a considerable increase in functional abilities reported in association with infliximab therapy.

Generally, there is a largely favourable risk/benefit ratio for infliximab. Infections, especially tuberculosis and opportunistic infections, are a potential concern. Although their incidence has not been significantly different from placebo groups in controlled clinical trials, postmarketing surveillance has noted 218 reported cases of tuberculosis to date and an increased risk of opportunistic infection. Pretreatment testing for tuberculosis is mandatory, and special caution is needed when signs of any infection occur in patients who are being treated with infliximab (or any anti-TNF treatment). The development of antinuclear and anti-DNA antibodies occurs during anti-TNF treatment; however, few clinically relevant cases of lupus-like syndromes have been reported. Another concern is the development of demyelinating disease. Malignancies have been carefully monitored, but the frequency has not exceeded that found in the overall RA population.

Patients with CHF should not receive anti-TNF agents. Elevation of liver enzymes and haematological abnormalities are very rare events, which should be monitored. The development of anaphylactic reactions has rarely been a serious problem. The use of infliximab without MTX for a period of time, followed by a cessation of therapy, may cause more problems when the therapy is restarted. Infusion reactions do occur, but can generally be managed.

Overall, infliximab treatment can be regarded as a major advantage in the therapy of several inflammatory diseases. Fewer problems can be expected when infliximab infusions are performed by experienced specialists.

Acknowledgements

The authors acknowledge the important contributions of P Geraghty, Centocor Inc., PA, USA.

Bibliography

1. LEE DM, WEINBLATT ME: Rheumatoid arthritis. *Lancet* (2001) 358:903-911.
2. BRAUN J, BOLLOW M, REMLINGER G *et al.*: Prevalence of spondylarthropathies in HLA-B27 positive and negative blood donors. *Arthritis Rheum.* (1998) 41(1):58-67.
3. CRANDALL WV, MACKNER LM: Infusion reactions to infliximab in children and adolescents: frequency, outcome and a predictive model. *Aliment Pharmacol. Ther.* (2003) 17:75-84.
4. ZINK A, LISTING J, KLINDWORTH C, ZEIDLER H: The national database of the German collaborative arthritis centre: I. Structures, aims and patients. *Ann. Rheum. Dis.* (2001) 60(3):199-206.
5. YELIN E, HENKE C, EPSTEIN W: The work dynamics of the person with rheumatoid arthritis. *Arthritis Rheum.* (1987) 30:507-512.
6. WOLFE F, KALEB M: Work disability in a national sample of RA patients. *Arthritis Rheum.* (2002) 46(9 Suppl.):S90.
7. PINCHBECK BR *et al.*: Economic impact of inflammatory bowel disease in Alberta. *Can. J. Gastroenterol.* (1988) 2:53-67.
8. BRAUN J, SIEPER J: Therapy of ankylosing spondylitis and other spondyloarthritides: established medical treatment, anti-TNF-α therapy and other novel approaches. *Arthritis Res.* (2002) 4:307-321.
9. BRAUN J, VAN DER HEIJDE D, DOUGADOS M *et al.*: Staging of patients with ankylosing spondylitis: a preliminary proposal. *Ann. Rheum. Dis.* (2002) 61(Suppl. 3):iii19-iii23.
10. BRAUN J, PINCUS T: Mortality, course of disease and prognosis of patients with ankylosing spondylitis. *Clin. Exp. Rheumatol.* (2002) 20(6) (Suppl. 28):S16-22.
11. SCHOELMERICH J: Future developments in diagnosis and treatment of inflammatory bowel disease. *Hepatogastroenterology* (2000) 47(31):101-114.
12. SCALLON BJ, MOORE MA, TRINH H, KNIGHT DM, GHRAYEB J: Chimeric anti-TNF-alpha monoclonal antibody cA2 binds recombinant transmembrane TNF-alpha and activates immune effector functions. *Cytokine* (1995) 7:251-259.
13. SCALLON B, CAI A, SOLOWSKI N *et al.*: Binding and functional comparisons of two types of tumor necrosis factor antagonists. *J. Pharmacol. Exp. Ther.* (2002) 301:418-426.
14. NIKOLAUS S, RAEDLER A, KÜHBACHER T, SFIKAS N, FÖLSCH UR, SCHREIBER S: Mechanisms in failure of infliximab for Crohn's disease. *Lancet* (2000) 356:1475-1479.
15. ESTERS N, VERMEIRE S, JOOSSENS S *et al.*: Serological markers for prediction of response to anti-tumor necrosis factor treatment in Crohn's disease. *Am. J. Gastroenterol.*(2002) 97:1458-1462.
16. KAVANAUGH A, ST CLAIR EW, MCCUNE WJ, BRAAKMAN T, LIPSKY P: Chimeric anti-tumor necrosis factor-α monoclonal antibody treatment of patients with rheumatoid arthritis receiving methotrexate therapy. *J. Rheumatol.* (2000) 27:841-850.
17. PHYSICIAN'S DESK REFERENCE (PDR): Remicade® (Centocor) infliximab recombinant for IV injection. Medical Economic Press, Montvale NJ (2002).
18. MAINI RN, BREEDVELD FC, KALDEN JR *et al.*: Therapeutic efficacy of multiple intravenous infusions of anti-tumor necrosis factor [alpha] monoclonal antibody combines with low-dose weekly methotrexate in rheumatoid arthritis. *Arthritis Rheum.* (1998) 41(9):1552-1563.
19. MAINI RN, ST CLAIR EW, BREEDVELD F *et al.*: Infliximab (chimeric anti-tumour necrosis factor α monoclonal antibody) versus placebo in rheumatoid arthritis patients receiving concomitant methotrexate: a randomized Phase III trial. *Lancet* (1999) 354:1932-1939.
20. RUTGEERTS P, FEAGAN BG, LICHTENSTEIN GR *et al.*: Comparison of scheduled and episodic treatment strategies of infliximab in Crohn's disease. (manuscript in preparation).
21. REZAIAN MM: Do infliximab and methotrexate act synergistically in the treatment of rheumatoid arthritis? Comment on the article by Maini *et al.* *Arthritis Rheum.* (1999) 42(8):1779.
22. ELLIOT MJ, MAINI RN, FELDMANN M *et al.*: Randomised double-blind comparison of chimeric monoclonal antibody to tumor necrosis factor α (cA2) versus placebo in rheumatoid arthritis. *Lancet* (1994) 344:1105-1110.
23. PALEOLOG EM, HUNT M, ELLIOTT MJ, FELDMANN M, MAINI R, WOODY, JN: Deactivation of vascular endothelium by monoclonal anti-tumor necrosis factor α antibody in rheumatoid arthritis. *Arthritis Rheum.* (1996) 39(7):1082-1091.
24. TAK PP, TAYLOR PC, BREEDVELD FC *et al.*: Decrease in cellularity and expression of adhesion molecules by anti-tumor necrosis factor α monoclonal antibody treatment in patients with rheumatoid arthritis. *Arthritis Rheum.* (1996) 39(7):1077-1081.
25. LIPSKY P, ST. CLAIR W, KAVANAUGH A *et al.*: Long-term control of signs and symptoms of rheumatoid arthritis with chimeric monoclonal anti-TNF[alpha] antibody (infliximab) in patients with active disease on methotrexate. *Arthritis Rheum.* (1998) 41(9):S364.
26. ELLIOT MJ, MAINI RN, FELDMANN M *et al.*: Treatment of rheumatoid arthritis with chimeric monoclonal antibodies to tumor necrosis factor α. *Arthritis Rheum.* (1993) 36:1681-1690.
27. LIPSKY PE, VAN DER HEIJDE DM, ST CLAIR EW *et al.*: Infliximab and methotrexate in the treatment of rheumatoid arthritis. *N. Engl. J. Med.* (2000) 343(22):1594-1602.
28. KEYSTONE E, HAN C, KEENAN GF, VAN DER HEIJDE D, HARRIMAN G: Infliximab plus methotrexate prevents structural damage progression in rheumatoid arthritis patients independent of clinical response. *Arthritis Rheum.* (2001) 44(9 Suppl.):S81.
29. KAVANAUGH A, MAINI R, BREEDVELD F *et al.*: Subgroup analyses show consistent clinical benefit in the ATTRACT trial: anti-TNF[alpha] monoclonal antibody, infliximab, in RA patients on methotrexate. *Arthritis Rheum.* (1999) 42(9 Suppl.):S78.
30. SHERGY WJ, PHILLIPS HM, HUNT RE, HERNANDEZ J: Experience with commercial Remicade® (Infliximab) at a large community-based Rheumatology practice. *Arthritis Rheum.* (2001) 44(9 Suppl.):S81.
31. LIPSKY P, ST. CLAIR W, FURST D *et al.*: 54-Week clinical and radiographic results from the ATTRACT trial: a Phase III study of infliximab (Remicade™) in patients with active RA despite methotrexate. *Arthritis Rheum.* (1999) 42(9 Suppl.):S401.
32. VAN DER HEIJDE DM, LANDEWÉ RB, LIPSKY PE, MAINI RN: Radiological

progression rate at baseline predicts treatment differences: results from the ATTRACT trial. *Arthritis Rheum.* (2001) 44(9 Suppl.):S80.

33. ANTONI CE, KAVANAUGH A, MANGER B *et al.*: Responses to infliximab therapy in the ATTRACT trial assess with the disease activity score (DAS): clinical response measured by DAS28 at 102 weeks correlates with arrest of radiologic progression and shows higher response rate than ACR20 criteria. *Ann. Rheum. Dis.* (2001) 60(Suppl. I):170.
34. MAINI R, VAN DER HEIJDE D, SMOLEN J *et al.*: Week 102 clinical and radiologic results from the ATTRACT trial: a two year, randomized, controlled Phase III trial of infliximab in patients with active RA despite MTX. *Ann. Rheum. Dis.* (2001) 60(Suppl. I):169.
35. WONG JB, LIPSKY PE, MAINI R, PATEL K, VAN DER HEIJDE D, THE ATTRACT STUDY GROUP: Rapid radiographic progression in rheumatoid arthritis and clinical and radiographic benefits from infliximab: results from ATTRACT. *Arthritis Rheum.* (2002) 46(9 Suppl.):S337.
36. QUINN MA, CONAGHAN PG, KARIM Z *et al.*: Rapid suppression of synovitis with infliximab. Results from a double blind placebo-controlled study using infliximab and MRI synovial quantification in early RA. *Arthritis Rheum.* (2001) 44(12):2947.
37. ST CLAIR EW, WAGNER C, WANG B *et al.*: Pharmacokinetics of infliximab therapy for rheumatoid arthritis (RA). *Arthritis Rheum.* (2001) 44(9):S214.
38. LORENZ HM, ANTONI C, VALERIUS T *et al.*: *In vivo* blockade of TNF-α by intravenous infusion of a chimeric monoclonal TNF-α antibody in patients with rheumatoid arthritis. *J. Immunol.* (1996) 156:1646-1653.
39. CHARLES PJ, SMEENK RJ, DE JONG J, FELDMANN M, MAINI RN: Assessment of antibodies to double-stranded DNA induced in rheumatoid arthritis patients following treatment with infliximab, a monoclonal antibody to tumor necrosis factor [alpha]: findings in open-label and randomized placebo-controlled trials. *Arthritis Rheum.* (2000) 43(11):2383-2390.
40. TAYLOR PC, STEUER A, CHARLES P *et al.*: Early RA patients on infliximab therapy show significant changes in sonographic measures of joint vascularity and serum VEGF by 18 weeks. *Arthritis Rheum.* (2001) 44(9):S152.
41. EMERY P, SHERGY W, YOCUM D, BALA M, HARRIMAN G: Rapid onset of response with infliximab treatment in RA patients. *Arthritis Rheum.* (2001) 44(9 Suppl.):S82.
42. LORENZ HM, GRÜNKE M, HIERONYMUS T *et al.*: *In vivo* blockade of tumor necrosis factor-α in patients with rheumatoid arthritis: long-term effects after repeated infusion of chimeric monoclonal antibody cA2. *J. Rheumatol.* (2000) 27:304-310.
43. ISERN R, SHERGY W, SANDERS C *et al.*: Safety and tolerability of a rapid infusion of Remicade® (infliximab) in the treatment of rheumatoid arthritis. *Ann. Rheum. Dis.* (2001) 60(Suppl. I):170.
44. KAVANAUGH A, ANTONI CE, MALA M, HARRIMAN G, HAN C, VAN DER HEIJDE D. Clinical and radiographic measures of disease activity are significantly correlated with employment status in rheumatoid arthritis (RA): results from the ATTRACT study. *Arthritis Rheum.* (2001) 44(9 Suppl.):S221.
45. KAVANAUGH A, HAN C, BALA M, MARSTERS P: Successful treatment improves employability in patients with rheumatoid arthritis (RA): results from the ATTRACT study. *Ann. Rheum. Dis.* (2002) 61(Suppl. I):342.
46. KAVANAUGH A, KEENAN G, DE WOODY K, BAO W, HENDRICKS D: Long-term follow-up of patients treated with infliximab in all completed clinical trials. *Arthritis Rheum.* (2002) 46(9 Suppl.):S535.
47. KIMURA Y, IMMUNDO LF, LI SC *et al.*: High dose infliximab in the treatment of resistant systemic juvenile rheumatoid arthritis. *Arthritis Rheum.* (2001) 44(9):S272.
48. CHATUVREDI VP: Pilot study of chimeric monoclonal anti-tumor necrosis factor alpha anti-body (infliximab) with methotrexate in polyarticular juvenile idiopathic arthritis. *Arthritis Rheum.* (2002) 46(9 Suppl.):LB12.
49. MASATLIOGLU S, GOGUS F, CEVIRGEN D, SEYAHI E, HATEMI G, OZDOGAN H: Infliximab in the treatment of refractory juvenile idiopathic arthritis. *Arthritis Rheum.* (2002) 46(9 Suppl.):S481.
50. HONAKEN VEA, TYNJÄLÄ P, VÄHÄSALO P, LAHDENNE P: Infliximab in juvenile arthritis: 1-year follow-up. *Arthritis Rheum.* (2002) 46(9 Suppl.):S480.
51. SCHIMIZZI GF, PUETT DW, SNOW DH *et al.*: Feasibility of tapering use of concomitant therapies in patients receiving infliximab. *Arthritis Rheum.* (2001) 44(9 Suppl.):S82.
52. KIELY PD, JOHNSON DM: Infliximab and leflunomide combination therapy in rheumatoid arthritis: an open-label study. *Rheumatology* (2002) 41:631-637.
53. DUREZ P, NZEUSSEU JP, DUFOUR JP *et al.*: Treatment of severe refractory rheumatoid arthritis (RA) with a combination of infliximab (IFX) and azathioprine (AZA): one year results of an open pilot study. *Ann. Rheum. Dis.* (2002) 61(Suppl. I):201.
54. SHERGY W, HARSHBARGER JL, LEE W *et al.*: Commercial experience with rheumatoid arthritis (RA) patients switching from etanercept to infliximab. *Ann. Rheum. Dis.* (2002) 61(Suppl. I):178.
55. TAYLOR PC, PETERS AM, PALEOLOG E *et al.*: Reduction of chemokine levels and leukocyte traffic to joints by tumor necrosis factor α blockade in patients with rheumatoid arthritis. *Arthritis Rheum.* (2000) 43(1):38-47.
56. ULFGREN AK, ANDERSSON U, ENGSTRÖM M *et al.*: Systemic anti-tumor necrosis factor [alpha] therapy in rheumatoid arthritis down-regulates synovial tumor necrosis factor [alpha] synthesis. *Arthritis Rheum.* (2000) 43(11):2391-2396.
57. ANTONI CE, DECHANT C, HAENTZSCHEL H *et al.*: Safety and efficacy of infliximab in 263 patients with active rheumatoid arthritis (RA) despite methotrexate therapy: a German open label trial. *Arthritis Rheum.* (2001) 44(9 Suppl.):S84.
58. ANTONI CE, FURST D, MANGER B: Outcome of pregnancy in women receiving Remicade® (infliximab) for the treatment of Crohn's disease or rheumatoid arthritis. *Arthritis Rheum.* (2001) 44(9 Suppl.):S152.
59. ERKAN D, YAZICI Y, KULMAN I *et al.*: Changes in outcomes after 6 months of infliximab in rheumatoid arthritis (RA): a subgroup analysis of methotrexate-receiving and non-receiving patients. *Arthritis Rheum.* (2001) 44(9):S83.

60. MAKSYMOWYCH WP, MALLON C, SPADY B *et al.*: The Alberta capital health region infliximab in rheumatoid arthritis prospective observational inception cohort: efficacy, adverse events, and withdrawal. *Arthritis Rheum.* (2001) 44(9)S82.

61. WOLFE F, MICHAUD K, KEENAN G, CALLEGARI P, BALA M: Measurement of infliximab effectiveness in clinical practice. *Arthritis Rheum.* (2002) 46(9 Suppl.):S534.

62. VAN VOLLENHOVEN RF, BRANNEMARK S, LINDBLAD S, KLARESKOG L: Treatment with TNFα antagonists results in significant gradual increases in work-force participation: data from the STURE registry. *Arthritis Rheum.* (2002) 46(9 Suppl.):S535.

63. QUINN MA, CONAGHAN PG, GREENSTEIN A, KARIM Z, BROWN C, EMERY P: Sustained response in early poor prognosis RA after withdrawal of infliximab therapy. *Arthritis Rheum.* (2002) 46(9 Suppl.):LB03.

64. QUINN M: Rapid sustained improvement with TNF blockade in early rheumatoid arthritis: results from a double blind placebo-controlled study with MRI outcomes. *Arthritis Rheum.* (2002) 46(9 Suppl.):S519.

65. TAYLOR PC; STEUER A, GRUBER J *et al.*: Infliximab attenuates joint destruction in early RA patients with ultrasonographic markers of poor prognosis. *Arthritis Rheum.* (2002) 46(9 Suppl.):S334.

66. RIDLEY D, MORETA E: Does change of anti-TNF agents provide improved clinical response in rheumatoid arthritis? *Ann. Rheum. Dis.* (2002) 61(Suppl. I):184.

67. VAN DULLEMEN HM, VAN DEVENTER SJ, HOMMES DW *et al.*: Treatment of Crohn's disease with anti-tumor necrosis factor chimeric monoclonal antibody (cA2). *Gastroenterology* (1995) 109(1):129-135.

68. MCCABE RP, WOODY J, VAN DEVENTER S *et al.*: A multicenter trial of cA2 anti-TNF chimeric monoclonal antibody in patients with active Crohn's disease. *Gastroenterology* (1996) 110: A962.

69. TARGAN SR, HANAUER SB, VAN DEVENTER SJ *et al.*: A short-term study of chimeric monoclonal antibody cA2 to tumor necrosis factor (alpha) for Crohn's disease. *N. Engl. J. Med.* (1997) 337(15):1029-1035.

70. PRESENT DH, RUTGEERTS P, TARGAN S *et al.*: Infliximab for the treatment of fistulas in patients with Crohn's disease. *N. Engl. J. Med.* (1999) 340(18):1398-1405.

71. HANAUER SB, FEAGAN BG, LICHTENSTEIN GR *et al.*: Maintenance infliximab for Crohn's disease: the ACCENT I randomised trial. *Lancet* (2002) 359:1541-1548.

72. SANDS B, VAN DEVENTER S, BLANK M, MARSTERS P, TRAVERS S, ACCENT II TRIAL: Maintenance infliximab (Remicade®) is safe and effective in fistulizing Crohn's disease (CD): results from the ACCENT II trial. *Am. J. Gastroeneterol.* (2002) 97(9 Suppl.):S258.

73. RUTGEERTS P, D'HAENS G, TARGAN S *et al.*: Efficacy and safety of retreatment with anti-tumor necrosis factor antibody (infliximab) to maintain remission in Crohn's disease. *Gastroenterology* (1999) 117:761-769.

74. D'HAENS G, VAN DEVENTER S, VAN HOGEZAND R *et al.*: Endoscopic and histological healing with infliximab anti-tumor necrosis factor antibodies in Crohn's disease: a European multicenter trial. *Gastroenterology* (1999) 116(5):1029-1034.

75. BAERT FJ, D'HAENS GR, PEETERS M *et al.*: Tumor necrosis factor α antibody (infliximab) therapy profoundly down-regulates the inflammation in Crohn's ileocolitis. *Gastroenterology* (1999) 116:22-28.

76. RUTGEERTS P, MALCHOW H, VATN MH *et al.*: Mucosal healing in Crohn's disease patients is associated with reduction in hospitalization and surgeries. *Gastroenterology* (2002) 122(4):Abstract M2138.

77. RUTGEERTS P, VAN ASSCHE G, VAN DEVENTER S *et al.*: Infliximab maintenance treatment strategy results in mucosal healing in patients with Crohn's disease: *Gastroenterology* (2002) 122(4):A618.

78. FASANMADE AA, TAWADROS R, ZHU Y *et al.*: Comparative pharmacokinetics of single- and multiple-dose infliximab in Crohn's disease patients. *Gastroenterology* (2002) 122(4):A617-A618.

79. LICHTENSTEIN GR, BAO W, KEENAN G, OLSON A, HANAUER S, COLOMBEL JF: Infliximab treatment allows Crohn's disease to reduce or discontinue concomitant corticosteroid use: ACCENT I year results. *Gastroenterology* (2002) 122(4):A615.

80. LICHTENSTEIN DR, BALA M, HAN C, DEWOODY K, SCHAIBLE T: Infliximab improved quality of life in patients with Crohn's disease. *Inflamm. Bowel Dis.* (2002) 8(4):237-243.

81. FEAGAN B, YAN S, BALA M, BAO W, OLSON A, HANAUER S: Infliximab maintenance therapy improves health related quality of life (HRQL) of Crohn's disease patients over 54 weeks. *Gastroenterology* (2002) 122(4):A615.

82. FEAGAN B, BALA M, YAN S, HANAUER S: High rates of disability and unemployment in the moderate to severe Crohn's disease patients. *Gastroenterology* (2002) 122(4):A603.

83. LICHTENSTEIN GR, BALA M, YAN S: Achieving remission improves employment rate and normalizes quality of life in Crohn's disease patients. *Gastroenterology* (2002) 122(4):A613.

84. FEAGAN BG, YAN S, BALA M, THORNE N, VAN DEVENTER S, LICHTENSTEIN GR: High rates of disability and unemployment in patients with fistulizing Crohn's disease. *Am. J. Gastroenterol.* (2002) 97(9 Suppl.):S264.

85. COLOMBEL JF, RUTGEERTS P, YAN S *et al.*: Infliximab (Remicade®) maintenance treatment results in lower hospitalization rate in Crohn's disease patients. *Gastroenterology* (2002) 122(4):A613.

86. LICHTENSTEIN GR, YAN S, BALA M, KEENAN G, HANAUER SB: Sustained remission lowers the likelihood of hospitalization and surgery in patients with Crohn's disease. *Am. J. Gastroenterol.* (2002) 97(9 Suppl.):S259.

87. LICHTENSTEIN GR, FEAGAN BG, YAN S, BALA M, HARTMAN P, SANDS B: Maintenance treatment with infliximab reduces hospitalizations and surgeries/procedures in patients with fistulizing Crohn's disease. *Am. J. Gastroenterol.* (2002) 97(9 Suppl.):S264-265.

88. GEBOES K, RUTGEERTS P, WAGNER C *et al.*: Infliximab treatment results in the sustained reduction of inflammatory markers in the mucosa of patients with Crohn's disease. *Gastroenterology* (2002) 122(4):A150.

89. SANDS B, D'HAENS G, RUTGEERTS P *et al.*: Change in C-reactive protein correlates with change in endoscopic measures of disease activity in Crohn's disease patients treated with infliximab.

Gastroenterology (2002) 122(4):Abstract W1602.

90. COHEN RD, TSANG JF, HANAUER SB: Infliximab in Crohn's disease: first anniversary clinical experience. *Am. J. Gastroenterol.* (2000) 95(12):3469-3477.
91. ARNOTT ID, MCDONALD D, WILLIAMS A, GHOSH S: Clinical use of infliximab in Crohn's disease: the Edinburgh experience. *Aliment Pharmacol. Ther.* (2001) 15(10):1639-1646.
92. ASAKURA H, YAO T, MATSUI T *et al.*: Efficacy of treatment with chimeric monoclonal antibody (infliximab) to tumor necrosis factor-alpha for Crohn's disease in Japan: evaluation by rapid turnover proteins, and radiologic and endoscopic findings. *J. Gastroenterol. Hep.* (2001) 16(7):763-769.
93. SABATE JM, VILLAREJO J, LEMANN M, BONNET J, ALLEZ M, MODIGLIANI R: An open-label study of thalidomide for maintenance therapy in responders to infliximab in chronically active and fistulizing refractory Crohn's disease. *Aliment Pharmacol. Ther.* (2002) 16:1117-1124.
94. TEN HOVE T, VAN MONTFRANS C, PEPPELENBOSCG MP, VSN DEVENTER SJ: Infliximab treatment induces apoptosis of lamina propria T lymphocytes in Crohn's disease. *Gut* (2002) 50:206-211.
95. FARRELL RJ, SAMIR AS, PARAG J *et al.*: Clinical experience with infliximab therapy in 100 patients with Crohn's disease. *Am. J. Gastroenterol.* (2000) 95(12):3490-3497.
96. MORTIMORE M, GIBSON PR, SELBY WS, RADFORD-SMITH GL, FLORIN THJ: The infliximab user group: Early Australian experience with infliximab, a chimeric antibody against tumour necrosis factor-alpha, in the treatment of Crohn's disease; is its efficacy augmented by steroid-sparing immunosuppressive therapy? *Intern. Med. J.* (2001) 31(3):146-150.
97. HOMMES DW, VAN DE HEISTEEG BH, VAN DER SPEK M, BARTELSMAN JF, VAN DEVENTER SJ: Infliximab treatment for Crohn's disease: one-year experience in a Dutch academic hospital. *Inflamm. Bowel Dis.* (2002) 8(2):81-86.
98. KENNEDY P, ANDREYEV J, GAZZARD *et al.*: Infliximab for Crohn's disease: one unit's experience. *Gut* (2002) 50(Suppl. 2):71a.
99. SAMPLE C, BAILEY RJ, TODORUK D *et al.*: Clinical experience with infliximab for Crohn's disease: the first 100 patients in Edmonton, Alberta. *Can. J. Gastroenterol.* (2002) 16(3):165-170.
100. GLAZIER KD, SHIRODKAR MA, KOSA E, MIRZA ZK, GRIFFEL LH, DAS KM: Clinical experience with infliximab therapy in the initial 256 infusions in patients with Crohn's disease at the Crohn's and Colitis Center of New Jersey. *Am. J. Gastroenterol.* (2002) 97(9 Suppl.):S262.
101. RICART E, PANACCIONE R, LOFTUS EV, TREMAINE WJ, SANDBORN WJ: Successful management of Crohn's disease of the ileoanal pouch with infliximab. *Gastroenterology* (2001) 117:429-432.
102. GEYER AS, ANHALT GJ, NOUSARI HC: Effectiveness of infliximab in the treatment of refractory perineal cutaneous Crohn's disease. *Arch. Dermatol.* (2000) 136:459-460.
103. LICHTENSTEIN GR, OLSON A, BAO W, TRAVERS S, DIAMOND RH: Infliximab treatment does not result in an increased risk of intestinal strictures or obstruction in Crohn's disease patients: ACCENT I study results. *Am. J. Gastroenterol.* (2002) 97(9 Suppl.):S254-255.
104. WEINBERG AM, RATTAN S, LEWIS JD *et al.*: Strictures and response to infliximab in Crohn's disease. *Am. J. Gastroenterol.* (2002) 97(9 Suppl.):S255.
105. LODHAVIA PJ, SCHERL EJ: Efficacy of infliximab in the treatment of refractory pouchitis. *Am. J. Gastroenterol.* (2002) 97(9 Suppl.):S180.
106. PARSI MA, ACHKAR JP, BRZEZINSKI A, SHEN B, LASHNER B: Extra-intestinal manifestations of Crohn's disease respond to infliximab. *Am. J. Gastroenterol.* (2002) 97(9 Suppl.):S265.
107. FLEISHER MR, RUBIN S, LEVINE A, HOPKINS K: Infliximab in the treatment of Crohn's disease complicated by granuloma annulare. *Proceedings of the American Academy of Dermatology, New Orleans* (2002).
108. TESSNOW K, CHIAO E, PETERSON K, JAMIJAN C, RIES K: Safety and efficacy of infliximab therapy in HIV patients with Crohn's disease. *Gastroenterology* (2002) 122(4):A616.
109. HADIGAN C, BALDASSANO R, BRAEGGER CP: Pharamcokinetics of infliximab (anti-TNFα) in children with Crohn's disease: a multicenter trial. *J. Pediatr. Gastroenterol. Nutr.* (1999) 29:525.
110. FASANMADE AA, ZHU YW, WAGNER C, PENDLEY C, DAVIS HM Population pharmacokinetics of single dose infliximab in patients with Crohn's disease. *Clin. Pharamcol. Ther.* (2002) 71(2):P66.
111. VANDERHOOF JA, YOUNG RJ: Autoimmune enteropathy in a child: response to infliximab therapy. *J. Pediatr. Gastroenterol. Nutr.* (2002) 34:312-316.
112. VASILIASUSKAS EA, THOMAS DW, SCHAFFER S *et al.*: Collaborative experience of open-label infliximab in refractory pediatric Crohn's disease. *J. Pediatr. Gastroenterol. Nutr.* (1999) 29(4):525.
113. HYAMS JS, MARKOWITZ J, WYLLIE R: Use of infliximab in the treatment of Crohn's disease in children and adolescents. *J. Pediatr.* (2000) 137(2):192-196.
114. ESCHER JC, STOFF TJ, VAN DEVENTER SJ, VAN FURTH AM: Successful treatment of metastatic Crohn's disease with infliximab. *J. Pediatr. Gastroenterol. Nutr.* (2002) 34:420-423.
115. MAMULA P, MARKOWITZ JE, BROWN KA, HURD LB, PICCOLI DA, BALDASSANO RN: Infliximab as a novel therapy for pediatric ulcerative colitis. *J. Pediatr. Gastroenterol. Nutr.* (2002) 34:307-311.
116. KUGATHASAN S, LEVY MB, SAEIAN K *et al.*: Infliximab retreatment in adults and children with Crohn's disease: risk factors for the development of delayed severe systemic reaction. *Am. J. Gastroenterol.* (2002) 97(6):1408-1414.
117. COHEN SA, SARIPKIN CJ, SARIPKIN LM *et al.*: Successful i.v. infusion of anti tumor necrosis factor alpha (infliximab) therapy in a pediatric office setting. *Am. J. Gastroenterol.* (2002) 97(9 Suppl.):S257-258.
118. SERRANO MS, SCHMIDT-SOMMERFIELD E, KILBAUGH TJ, BROWN RF, UDALL JN, MANNICK EE: Use of infliximab in pediatric patients with inflammatory bowel disease. *Ann. Pharmacother.* (2001) 35:823-828.
119. RUBENSTEIN JH, CHONG RY, COHEN RD: Infliximab decreases resource use among patients with Crohn's disease.

J. Clin. Gastroenterol. (2002) 35(2):151-156.

120. KHAN MA: Update on spondyloarthropathies. *Ann. Intern. Med.* (2002) 136:896-907.
121. BRAUN J, SIEPER J: Commented glossary for rheumatic spinal diseases. *Ann. Rheum. Dis.* (1996) 55(1):76.
122. BRAUN J, XIANG J, BRANDT J *et al.*: Treatment of spondyloarthropathies with antibodies against tumour necrosis factor [alpha]: first clinical and laboratory experiences. *Ann. Rheum. Dis.* (2000) 59(Suppl. I):i85-i89.
123. BROWN MA, CRANE AM, WORDSWORTH BP: Genetic aspects of susceptibility, severity, and clinical expression in ankylosing spondylitis. *Curr. Opin. Rheumatol.* (2002) 14(4):354-360.
124. BROWN MA, LAVAL SH, BROPHY S, CALIN A: Recurrence risk modeling of the genetic susceptibility to ankylosing spondylitis. *Ann. Rheum. Dis.* (2000) 59(11):883-886.
125. KHAN MA, KAHN MK. Diagnostic value of HLA-B27 testing in ankylosing spondylitis and Reiter's syndrome. *Ann. Intern. Med.* (1982) 96:70-76.
126. VAN DEN LINDEN S, VALKENBURG HA, CATS A: Evaluation of diagnostic criteria for ankylosing spondylitis: a proposal for modification of the New York criteria. *Arthritis Rheum.* (1984) 27:361-368.
127. LAUTERMANN D, BRAUN J: Ankylosing spondylitis – cardiac manifestations. *Clin. Exp. Rheumatol.* (2002) 20(6 Suppl. 28):S11-S15.
128. BRAUN J, BOLLOW M, NEURE L *et al.*: Use of immunohistologic and *in situ* hybridization techniques in the examination of sacroiliac joint biopsy specimens from patients with ankylosing spondylitis. *Arthritis Rheum.* (1995) 38(4):499-505.
129. VAN DER HEIJDE D, BELLAMY N, CALIN A, DOUGADOS M, KHAN A, VAN DER LINDEN S: Preliminary core sets for endpoints in ankylosing spondylitis. *Arthritis Rheum.* (1997) 40(9 Suppl.):S62.
130. ANDERSON JJ, BARON G, VAN DER HEIJDE D, FELSON DT, DOUGADOS M: Ankylosing spondylitis assessment group preliminary definition of short-term improvement in ankylosing spondylitis. *Arthritis Rheum.* (2001) 44(8):1876-1886.
131. GARRETT S, JENKINSON TR, KENNEDY LG, WHITELOCK HC, GAISFORD P, CALIN A: A new approach to defining disease status in ankylosing spondylitis. The Bath ankylosing spondylitis disease activity index. *J. Rheumatol.* (1994) 21:2286-2291.
132. CALIN A, GARRETT S, WHITELOCK HC *et al.*: A new approach to defining functional ability in ankylosing spondylitis. The Bath ankylosing spondylitis functional index. *J. Rheumatol.* (1994) 21:2281-2285.
133. JENKINSON TR, MALLORIE PA, WHITELOCK HC, KENNEDY LG, GARRETT S, CALIN A: Defining spinal mobility in ankylosing spondylitis. *J. Rheumatol.* (1994) 21:1694-1698.
134. MACKAY K, MACK C, BROPHY S, CALIN A: The Bath ankylosing spondylitis radiology index (BASRI): a new, validated approach to disease assessment. *Arthritis Rheum.* (1998) 41(12):2263-2270.
135. BRANDT J, HAIBEL H, CORNELY D *et al.*: Successful treatment of active ankylosing spondylitis with the anti-tumor necrosis factor [alpha] monoclonal antibody infliximab. *Arthritis Rheum.* (2000) 43(6):1346-1352
136. VAN DEN BOSCH F, KRUITHOF E, BAETEN D, DE KEYSER F, MIELANTS H, VEYS EM: Effects of a loading dose regimen of three infusions of chimeric monoclonal antibody to tumor necrosis factor α (infliximab) in spondyloarthropathy: an open pilot study. *Ann. Rheum. Dis.* (2000) 59:428-433.
137. BRAUN J, BRANDT J, LISTING J *et al.*: Treatment of active ankylosing spondylitis with infliximab: a randomised controlled multicenter trial. *Lancet* (2002) 359:1187-1193.
138. VAN DEN BOSCH F, KRUITHOF E, BAETEN D *et al.*: Randomized double-blind comparison of chimeric monoclonal antibody to tumor necrosis factor α (infliximab) versus placebo in active spondylarthropathy. *Arthritis Rheum.* (2002) 46(3):755-765.
139. BRAUN J, BRANDT J, LISTING J *et al.*: Long-term efficacy and safety of infliximab in the treatment of ankylosing spondylitis. *Manuscript in preparation.*
140. BRAUN J, BARALIAKOS X, GOLDER W *et al.*: Ankylosing spondylitis (AS) – development and evaluation of a spinal scoring system (ASspiMRI) using magnetic resonance imaging (MRI) in patients with active disease. *Arthritis Rheum.* (2002) 46(9 Suppl.):S426.
141. BRAUN J, BARALIAKOS X, GOLDER W *et al.*: Improvement of spinal inflammation in ankylosing spondylitis (AS) by infliximab therapy as assessed by magnetic resonance imaging (MRI) using a novel evaluated spinal scoring system. *Arthritis Rheum.* (2002) 46(9 Suppl.):S426.
142. VAN DEN BOSCH, KRUITHOF E, BAETEN D: Safety and efficacy of a retreatment regimen of 10 mg/kg infliximab every 14 weeks in patients with active spondylarthropathy. *Arthritis Rheum.* (2002) 46(9 Suppl.):S430.
143. BAETEN D, KRUITHOF E, VAN DEN BOSCH F *et al.*: Immunomodulatory effects of anti-tumor necrosis factor αtherapy on synovium in spondylarthropathy. *Arthritis Rheum.* (2001) 44(1):186-195.
144. BREBAN M, VIGNON E, CLAUDEPIERRE P *et al.*: Efficacy of infliximab in severe refractory ankylosing spondylitis (AS). Results of a 6 month follow-up open-label study. *Arthritis Rheum.* (2001) 44(9 Suppl.) A222.
145. STONE M, SALONEN D, LAX M, PAYNE U, LAPP V, INMAN R: Clinical and imaging correlates of response to treatment with infliximab in patients with ankylosing spondylitis. *J. Rheumatol.* (2001) 28:1605-1614.
146. KRUITHOF E, VAN DEN BOSCH F, BAETEN D *et al.*: Repeated infusions of infliximab, a chimeric anti-TNF-α monoclonal antibody, in patients with active spondyloarthropathy: one year follow up. *Ann. Rheum. Dis.* (2002) 61:207-212.
147. MAKSYMOWYCH WP, JHANGRI GS, LAMBERT RG *et al.*: Infliximab in ankylosing spondylitis: a prospective observational inception cohort analysis of efficacy and safety. *J. Rheumatol.* (2002) 29(5):959-965.
148. SIDIROPOULOS P, VOUDOURIS K: Infliximab in seronegative spondylarthritides (SpA). *Ann. Rheum. Dis.* (2002) 61(Suppl. I):306.
149. BOKI KA, CHEROUVIM EP, MOUTSOPOULOS HM: TNF-α blockade with infliximab in patients with active and refractory ankylosing spondylitis. Preliminary results of an open-label study. *Ann. Rheum. Dis.* (2002) 61(Suppl. I):308.
150. BOEGER CA, WITTWER H, SCHATTENKIRCHNER M,

KELLNER H, KELLNER W: Treatment of ankylosing spondylitis with infliximab. *Ann. Rheum. Dis* (2001) 60:1159-1160.

151. KAISER MJ, SANY J: Efficacy of infliximab (Remicade®) in the treatment of spondyloarthropathies: Two case reports. *Joint Bone Spine* (2001) 68:525-527.
152. D'AGOSTINO MA, BREBAN M, SAID-NAHAL R, DOUGADOS M: Refractory inflammatory heel pain in spondylarthropathy: a significant response to infliximab documented by ultrasound. *Arthritis Rheum.* (2002) 46(3):840-842.
153. HADI A, HICKLING P, BROWN M, AL-NAHHAS A: Scintigraphic evidence of effect of infliximab on disease activity in ankylosing spondylitis. *Rheumatology* (2002) 41:114-116.
154. ZOU J, RUDWALEIT M, BRANDT J *et al.*: Comparison of the non-specific and antigen-specific T-cell cytokine response during treatment with infliximab or etanercept in ankylosing spondylitis. *Arthritis Rheum.* (2002) 46(9 Suppl.):S381.
155. KOROKNAY A, STIEGER G, BRAUN J *et al.*: Analysis of SNP's of the TNFA promoter/enhancer region in patients with ankylosing spondylitis (AS) treated with infliximab. *Arthritis Rheum.* (2002) 46(9 Suppl.):S434.
156. GU J, BAETEN D, RIHL M *et al.*: Gene transcripts in the synovia of spondylo-arthropathy (SpA) are clustered into statistical subgoups, each with a unique potential in function, as well as in response to infliximab therapy. *Arthritis Rheum.* (2002) 46(9 Suppl.):S435.
157. TOMERO EG, CARMONA L, GONZÁLEZ -ALVARO *et al.*: Infliximab in secondary amyloidosis complicating inflammatory arthropathies. *Arthritis Rheum.* (2002) 46(9 Suppl.):S70.
158. KRUITHOF E, BAETEN D, VAN DEN BOSCH F, MIELANTS H, VEYS EM, DE KEYSER F: Modulation of vascularity and expression of adhesion molecules by TNFalpha blockade in spondyloarthropathy: histologic and serologic evaluation in a placebo-controlled trial. *Arthritis Rheum.* (2002) 46(9 Suppl.):S430.
159. MARZO-ORTEGA H, MC GONAGLE D, O'CONNOR P, EMERY P: Efficacy of etanercept in the treatment of the entheseal pathology in resistant spondylarthropathy: a clinical and magnetic resonance imaging study. *Arthritis Rheum.* (2001) 44(9):2112-17.
160. MARZO-ORTEGA H, MCGONAGLE D, O'CONNOR P, EMERY P: Efficacy of etanercept for treatment of Crohn's related spondylarthritis but not colitis. *Ann. Rheum. Dis.* (2003) 62(1):74-76.
161. GORMAN J, SACK KE, DAVIS JC JR: Treatment of ankylosing spondylitis by inhibition of tumor necrosis factor α. *N. Engl. J. Med.* (2002) 346(18):1349-1356.
162. BRANDT J, KARIOUZOV A, LISTING J *et al.*: Six months results of a German double-blind placebo controlled, phase-III clinical trial of etanercept in active ankylosing spondylitis. *Arthritis Rheum.* (2002) 46(9 Suppl.):S429.
163. GOOSSENS PH, VERBURG RJ, BREEDVELD FC: Remission of Behçet's syndrome with tumour necrosis factor α blocking therapy. *Ann. Rheum. Dis.* (2001) 60:637.
164. HASSARD PV, BINDER SW, NELSON V, VASILIASUSKAS EA: Anti-tumor necrosis factor monoclonal antibody therapy for gastrointestinal Behçet's disease: a case report. *Gastroenterology* (2001) 120(4):995-999.
165. ROBERTSON LP, HICKLING P: Treatment of recalcitrant orogenital ulceration of Behçet's syndrome with infliximab. *Rheumatology* (2002) 40:473-474.
166. TRAVIS SP, CZAJKOWSKI M, GOVERN DP, WATSON RG, BELL AL: Treatment of intestinal Behçet's syndrome with chimeric tumour necrosis factor α antibody. *Gut* (2001) 49:725-728.
167. ROZENBAUM M, ROSNER I, PORTNOY E: Remission of Behçet's syndrome with TNFα blocking agent. *Ann. Rheum. Dis.* (2002) 61:283-284.
168. TRIOLO G, VADALÁ M, ACCARDO-PALUMBO A *et al.*: Anti-tumour necrosis factor monoclonal antibody treatment for ocular Behçet's disease. *Ann. Rheum. Dis.* (2002) 61:560-561.
169. SINGRI P, WEST DP, GORDON KB: Biologic therapy for psoriasis: the new therapeutic frontier. *Arch. Dermatol.* (2002) 138:657-663.
170. CLEGG DO, REDA DJ, MEJIAS E *et al.* Comparison of sulfasalazine and placebo in the treatment of psoriatic arthritis: a Department of Veterans Affairs cooperative study. *Arthritis Rheum.* (1996) 39(12):2013-2020.
171. CHAUDHARI U, ROMANO P, MULCAHY LD, DOOLEY LT, BAKER DG, GOTTLIEB AB: Efficacy and safety of infliximab monotherapy for plaque-type psoriasis: a randomised trial. *Lancet* (2001) 357:1842-1847.
172. GOTTLIEB AB, MASUD S, RAMAMURTHI R: Infliximab monotherapy normalizes keratinocyte differentiation and decreases inflammation in skin biopsies from patients with moderate to severe plaque psoriasis. *Proceedings from the American Academy of Dermatology, New Orleans* (2002).
173. GOTTLIEB AG, ROMANO P, CHAUDHARI U, BAKER D, LI S: Infliximab prevents relapse of moderate to severe psoriasis in responding patients. *Proceedings of the World Congress of Dermatology, Paris, France* (2002):P1973.
174. ANTONI A, OGILVIE A, LUEFTL M *et al.*: The infliximab multinational psoriatic arthritis controlled trial (IMPACT). *Arthritis Rheum.* (2002) 46(9 Suppl.):S381.
175. MEASE P, KIVITZ A, BURCH F, SIEGEL E, COHEN S, BURGE D: Improvement in disease activity in patients with psoriatic arthritis receiving etanercept (Enbrel®): results of a Phase III multicenter clinical trial. *Arthritis Rheum.* (2001) 44(9 Suppl.):S90.
176. OGILVIE AL, ANTONI C, DECHANT C *et al.*: Treatment of psoriatic arthritis with antitumour necrosis factor-α antibody clears skin lesions or psoriasis resistant to treatment with methotrexate. *Br. J. Dermatol.* (2001) 144:587-589.
177. SCHOPF RE, AUST H, KNOP J: Treatment of psoriasis with the chimeric monoclonal antibody against tumor necrosis factor α, infliximab. *J. Am. Acad. Dermatol.* (2002) 46:886-891.
178. CHEROUVIM EP, BOKI KA, MOUTSOPOULOS HM: Efficacy of infliximab in active and refractory psoriatic arthritis. Preliminary results of an open-label study. *Ann. Rheum. Dis.* (2002) 61(Suppl. I):308.
179. FELETAR MH, BROCKBANK JE, SCHENTAG CT, LAPP V, GLADMAN DD: Treatment of recalcitrant psoriatic arthritis patients with infliximab – a 12 month observational study of 16 patients. *Arthritis Rheum.* (2002) 46(9 Suppl.):SY15.
180. BOLCE R, THOMPSON J, STEVENS MP: Treatment of psoriatic

arthritis with infliximab in a small office-based rheumatology practice. *Arthritis Rheum.* (2001) 44(9 Suppl.):S121.

181. STEVENS MP, LEE WW, MAKAROWSKI WS: Infliximab in the treatment of psoriatic arthritis and psoriasis. *Proceedings of the World Congress of Dermatology, Paris, France* (2002):P2029.

182. FLEISCHMANN R, SEVENS MP, BROOKS MS: Infliximab in the treatment of psoriasis and psoriatic arthritis. *Proceedings from the American Academy of Dermatology, New Orleans* (2002).

183. DROSOU A, KIRSNER R, WELSH E: Use of infliximab, an anti-TNF alpha antibody in dermatologic diseases. *Proceedings of the World Congress of Dermatology, Paris, France* (2002).

184. KIRBY B, MARSLAND AM, CARMICHAEL AJ, GRIFFITHS CE: Successful treatment of severe recalcitrant psoriasis with combination infliximab and methotrexate. *Clin. Exp. Dermatol.* (2001) 26:27-29.

185. RUZICKA T, MEGAHED M, GREWE *et al.*: Psoriasis arthropathica: Erfolgreiche Therapie mit einem TNF-alpha Antikörper (infliximab). *Z. Hautkr.* (2001) 76:232-253.

186. TAN MH, GORDON M, LEBWOHL O, GEORGE J, LEBWOHL MG: Improvement of pyoderma gangrenosum and psoriasis associated with Crohn's Disease with anti-tumor necrosis factor α monoclonal antibody. *Arch. Dermatol.* (2001) 137:930-933.

187. O'QUINN RP, MILLER JL: The effectiveness of tumor necrosis factor α antibody (infliximab) in treating recalcitrant psoriasis. *Arch. Dermatol.* (2002) 138:644-648.

188. BRAY VJ, HUFSTUTTER JE, SCHWARTZMAN S: Emerging role of infliximab (Remicade®) in psoriatic arthritis patients resistant to disease-modifying antirheumatic drugs: case studies. *Arthritis Rheum.* (2001) 46(9 Suppl.):S121.

189. MANG R, STEGE H, RUZICKA T, KRUTMANN J: Response of severe psoriasis to infliximab. *Dermatology* (2002) 204:156-157.

190. MENTER A, JEROME S, CUSH JM: Successful treatment of pediatric psoriasis with infliximab. *Proceedings from the American Academy of Dermatology, New Orleans* (2002).

191. BATRES LA, MAMULA P, BALDASSANO RN: Resolution of severe peristomal pyoderma gangrenosum with infliximab in a child with Crohn's disease. *J. Pediatr. Gastroenterol. Nutr.* (2002) 34:558-560.

192. RUGIERO M: Infliximab for treatment of pyoderma gangrenosum associated with inflammatory bowel disease. (manuscript in preparation).

193. FRIEDMAN S, MARION JF, SCHERL E, RUBIN PH, PRESENT DH: Intravenous cyclosporine in refractory pyoderma gangrenosum complicating inflammatory bowel disease. *Inflamm. Bowel Dis.* (2001) 7(1):1-7.

194. PLEVY S, VALENTINE J, FLEISHER MR, LICHTENSTEIN GR: Successful treatment of IBD-associated pyoderma gangrenosum with infliximab. *Proceedings from the American Academy of Dermatology, New Orleans* (2002).

195. LÍMOVÁ M: Treatment of pyoderma gangrenosum with intravenous infliximab. *Proceedings from the American Academy of Dermatology, New Orleans* (2002).

196. FOSTER E, NGUYEN KK, BOLCE R, PRINDVILLE TP: Cutaneous manifestations of inflammatory bowel disease improve with infliximab. *Gastroenterology* (2002) 122(4):A618.

197. ACHKER JP, BRZEZINSKI A, DELANEY C, HULL T, LASHNER B: Successful treatment of peristomal pyoderma gangrenosum with infliximab. *Am. J. Gastroenterol.* (2002) 97(9 Suppl.):S261.

198. SMITH SR, ROSENBAUM JT: Management of uveitis. *Arthritis Rheum.* (2002) 46(2):309-318.

199. KRUITHOF E, KESTELYN P, ELEWAUT C: Successful use of infliximab in a patient with treatment resistant spondyloarthopathy related uveitis. *Ann. Rheum. Dis.* (2002) 61:470.

200. SFIKAKIS PP, THEODOSSIADIS PG, KATSIARI CG, KAKLAMANIS P, MARKOMICHELAKIS NN: Effect of infliximab on sight-threatening panuveitis in Behçet's disease. *Lancet* (2001) 358:295-296.

201. EL-SHABRAWI Y, HERMANN J, FRANZENS K: Anti-TNFα therapy with infliximab as an alternative to corticosteroids in the treatment of HLA B27 associated acute anterior uveitis (AAU). *Ann. Rheum. Dis.* (2001) 60(Suppl. I):144.

202. EL-SHABRAWI Y, HERMANN J: Anti-tumor necrosis factor-alpha therapy with infliximab as an alternative to corticosteroids in the treatment of human leukocyte antigen B27-associated acute anterior uveitis. *Ophthalmology* (2002) 109(12):2342-2346.

203. FRIES W, GIOFRÉ MR, CATANOSO M, LO GULLO R: Treatment of acute uveitis associated with Crohn's disease and sacroileitis with infliximab. *Am. J. Gastroenterol.* (2002) 97(2):499-500.

204. SCHIMIZZI G: Infliximab treatment in uveitis: a case report. *Proceedings of the Federation of Clinical Immunology, San Francisco, California, USA* (2002).

205. HONKANEN V, LAPPI M, KOSKINEN L, LINDAHL P, LAHDENNE P Infliximab treatment in the refractory chronic uveitis of juvenile idiopathic arthritis (JIA). *Arthritis Rheum.* (2001) 44(9 Suppl.):S292.

206. PATO E, ABÁSOLO L, MACARRÓN *et al.*: Treatment of refractory posterior uveitis with anti-TNF-α (infliximab). *Arthritis Rheum.* (2001) 44(9 Suppl.):S90.

207. SFIKAKIS PP, KAKLAMANIS P, KATSIARI CG, ELEZOGLOU A, THEODOSSIADIS P, MARKOMICHELAKIS N: Successful treatment of ocular relapse in patients with Behçet's disease with a single infusion of the anti-TNFagent infliximab. *Arthritis Rheum.* (2002) 46(9 Suppl.):S181.

208. SCHWARTZMAN S, FLYNN T, BARINSTEIN L, GARTNER S, ONEL K: Infliximab therapy for resistant uveitis. *Arthritis Rheum.* (2002) 46(9 Suppl.):S326.

209. EL-SHABRAWI Y, HERMANN J: Case series of selective anti-tumor necrosis factor alpha therapy using infliximab in patients with nonresponsive chronic HLA-B27-associated anterior uveitis: comment on the articles by Brandt *et al. Arthritis Rheum.* (2002) 46(10):2821-2822.

210. BRAUN J, SIEPER J: Reply: Case series of selective anti-tumor necrosis factor alpha therapy using infliximab in patients with nonresponsive chronic HLA-B27-associated anterior uveitis: comment on the articles by Brandt *et al. Arthritis Rheum.* (2002) 46(10):2822-2824.

211. SANDS BE, TREMAINE WJ, SANDBORN WJ *et al.*: Infliximab in the treatment of severe, steroid-refractory ulcerative colitis: a pilot study. *Inflamm. Bowel Dis.* (2001) 7(2):83-88.

212. FLEISHER MR, RUBIN SD, LEVINE AE: Infliximab in the treatment of

steroid-naïve ulcerative colitis. *Am. J. Gastroenterol.* (2001) 96(9 Suppl.):S291-S292.

213. CHEY W, KUNZE GY, SHAH AN: Observations on therapeutic effect of infliximab on ulcerative colitis. *Am. J. Gastroenterol.* (2001) 96(9 Suppl.):S288.
214. SU C, SALZBERG BA, LEWIS JD *et al.*: Efficacy of anti-tumor necrosis factor therapy in patients with ulcerative colitis. *Am. J. Gastroenterol.* (2002) 97(10):2577-2584.
215. ANKER SD, COATS AJS: How to RECOVER from RENAISSANCE? The significance of the results of RECOVER, RENAISSANCE, RENEWAL and ATTACH. *Int. J. Cardiol.* (2002) 86:123-130.
216. KEANE J, GERSHON SK, BRAUN MM: Tuberculosis and treatment with infliximab. *N. Engl. J. Med.* (2002) 346(8):623-626.
217. BROWN SL, GREENE MH, GERSHON SK, EDWARDS ET, BRAUN MM: Tumor necrosis factor antagonist therapy and lymphoma development. *Arthritis Rheum.* (2002) 46(12):3151-8.
218. URBANSKY K, SANFORD T, MICHAUD K, KEENAN G, CALLEGARI P, WOLFE F: Safety data from a registry of patients receiving infliximab – preliminary report after 1 year. *Arthritis Rheum.* (2002) 46(9 Suppl.):S573.
219. GOTTENBERG JE, MERLE-VINCENT F, DOUGADOS M *et al.*: Anti-TNFα therapy in 12 patients with AA amyloidosis: a follow-up report of tolerance and efficacy. *Arthritis Rheum.* (2002) 46(9 Suppl.):S71.
220. ORTIZ-SANTAMARIA V, VALLS-ROC M, SANMARTÍ M *et al.*: Treatment of secondary amyloidosis with infliximab. *Arthritis Rheum.* (2002) 46(9 Suppl.):S71.
221. FIECHTNER J: Treatment of primary biliary cirrhosis with Remicade® (infliximab). *Arthritis Rheum.* (2001) 44(9 Suppl.):S222.
222. GILLETT HR, ARNOTT ID, MCINTYRE M *et al.*: Successful infliximab treatment for steroid-refractory celiac disease: a case report. *Gastroenterology* (2002) 122:800-805.
223. FISKE S, SPIRA RS: Infliximab in the management of erythema nodosum. *Proceedings of the World Congress of Dermatology, Paris, France* (2002):P1061.
224. CANTINI F, NICCOLI L, SALVARANI C, PADULA A, OLIVIERI I: Treatment of longstanding active cell arteritis with infliximab: report of four cases. *Arthritis Rheum.* (2001) 44(12):2933-2935.
225. AIRÓ P, ANTONIOLI AM, VIANELLI M, TONIATI P: Anti-tumour necrosis factor treatment with infliximab in a case of giant cell arteritis resistant to steroid and immunosuppressive drugs. *Rheumatology* (2002) 41:347-349.
226. BOH E, SHERROD J, SAFAH H, HUYNH-LE T: Successful use of infliximab in a patient with graft versus host disease. *Proceedings of the World Congress of Dermatology, Paris, France* (2002):P1649.
227. RIVKINA AM, STUMP LS: Infliximab in graft-versus-host disease. *Am. J. Health Sys. Pharm.* (2002) 59(13):1271-1275.
228. SULLIVAN TP, WELSH E, KERDEL FA, BURDICK AAE, KIRSNER RS: Infliximab for hidradenitis suppurativa. *Proceedings of the World Congress of Dermatology, Paris, France* (2002).
229. WEISS JE, EBERHARD BA, GOTTLIEB BS: Infliximab as a novel therapy for refractory Kawasaki disease (KD). *Arthritis Rheum.* 46(9 Suppl.):S310.
230. SCOTT JW, FRANCOIS A, HUYNH-LE T, MAGNUS JH: Experience with infliximab in the treatment of mixed connective tissue disease/lupus. *Ann. Rheum. Dis.* (2002) 61(Suppl. I):97.
231. HUMENIUK JM, GLIPTIS EG: Infliximab in the treatment of panniculitis. *Proceedings of the World Congress of Dermatology, Paris, France* (2002):P1679.
232. NOSSENT HC, BAKLAND G, ASLAKSEN HK, OLSEN G, NORDVAG BY: Methylprednisolone pulse therapy versus infliximab in the treatment of severe flares of chronic polyarthritis. *Arthritis Rheum.* (2001) 44(9 Suppl.):S369.
233. WOUTERS C, CORNILLIE F, BILLIAU AD: Anti-TNFα Therapy for systemic juvenile idiopathic arthritis with polyarthritis. *Ann. Rheum. Dis.* (2002) 61(Suppl. I):241.
234. EHRESMANN G: Infliximab in the treatment of polychondritis. *Arthritis Rheum.* (2002) 46(9 Suppl.):S160.
235. GARCIA-PORRUA A, GONZÁLEZ-GAY MA: Successful treatment of refractory mononeuritis multiplex secondary to rheumatoid arthritis with anti-tumor necrosis factor a monoclonal antibody infliximab. *Rheumatology* (2002) 41:234-235.
236. GAYLIS N: Infliximab in the treatment of an HIV-positive patient with Reiter's syndrome. (manuscript submitted).
237. KELLNER H, KROETZ M, SCHATTENKIRCHNER M, KELLNER W: Successful therapy of sacroiliitis in AS patients by intraarticular infection of infliximab. *Arthritis Rheum.* (2002) 46(9 Suppl.):S431.
238. BAUGHMAN RP, LOWER EE: Infliximab for refractory sarcoidosis. *Sarcoidosis Vasc. Diffuse Lung Dis.* (2001) 18:70-74.
239. YEE, AM, POCHAPIN MB: Treatment of complicated sarcoidosis with infliximab anti-tumor necrosis factor-[alpha] therapy. *Ann. Intern. Med.* (2001) 135(1):27-31.
240. FAHEY SM, BOLSTER M, SILVER RM, JUDSON M: Treatment of refractory sarcoidosis with infliximab (Remicade). *Arthritis Rheum.* (2002) 46(9 Suppl.):S323.
241. SWEISS NJ, ELLMAN MH, CURRAN JJ, PARK C: TNF- Inhibition as novel treatment for refractory acidosis. *Arthritis Rheum.* (2002) 46(9 Suppl.):S324.
242. STEINFELD SD, DEMOIS P, SALMON I, ROBERT K, APPELBOOM T: Infliximab in patients with primary Sjögren's syndrome: a pilot study. *Arthritis Rheum.* (2001) 44(10):2371-2375.
243. KRAETSCH HG, ANTONI C, KALDEN JR, MANGER B: Successful treatment of a small cohort of patients with adult onset of Still's disease with infliximab: first experience. *Ann Rheum Dis* (2001) 60(Suppl. 3):iii55-iii57.
244. HUFSTUTTER JE, SIENKNECHT CW: Treatment of resistant adult Still's disease with infliximab – report of two cases. *Arthritis Rheum.* (2002) 46(9 Suppl.):S326.
245. LAMPRECHT P, ARBACH O, VOSWINKEL *et al.*: Induction of remission with infliximab in refractory Wegener's granulomatosis (WG) – an update on the follow-up of 6 patients. *Arthritis Rheum.* (2002) 46(9 Suppl.):S186.
246. BRESCIA AM, MC ILVAIN-SIMPSON G, ROSE CD: Infliximab therapy for steroid-dependent early onset sarcoid arthritis and blau syndrome. *Arthritis Rheum.* (2002) 46(9 Suppl.):S313.
247. FORSTER A, HÜRLIMANN D, RUSCHITZKA F *et al.*: Vascular endothelial dysfunction in rheumatoid arthritis is improved by anti-TNFα

treatment with infliximab. *Arthritis Rheum.* (2002) 46(9 Suppl.):S511.

248. VOIGTLAENDER C, LÜFTL M, SCHULER G, MERTL M: Infliximab (anti-tumor necrosis factor α antibody), a novel, highly effective treatment of recalcitrant subcorneal pustular dermatosis (Sneddon-Wilkinson disease). *Arch. Dermatol.* (2001) 137:1571-1574.

Affiliation

Prof. Dr J Braun[†1] & Prof. Dr J Sieper[2]
[†]Author for correspondence
[1]Rheumazentrum Ruhrgebiet, Landgrafenstr. 15, 44652 Herne, Germany
Tel: +49 23 2559 2131; Fax: +49 23 2559 2136;
E-mail: J.Braun@rheumazentrum-ruhrgebiet.de
[2]Department of Gastroenterology and Rheumatology, Klinikum Benjamin Franklin, Free University Berlin, 12200 Berlin, Germany
Tel: +49 30 8445 4547;
Fax: +49 30 8445 4582;
E-mail: hjsieper@zedat.fu-berlin.de

Appendices

Table 1. Controlled studies in rheumatoid arthritis using infliximab – primary endpoint efficacy.

Citation	n	Dosage (mg/kg)	Duration (weeks)	Ref.
Elliott (1993)	20	20	8	[26]
Elliott (1994)	73	1, 10	4	[22]
Paleolog (1996)	73	1, 10	4	[23]
Tak P (1996)	14	10, 20	4	[24]
Maini (1998)	101	1, 3, 10	26	[18]
Lipsky (1998)	19	10	40	[25]
Maini (1999)	428	3, 10	30	[19]
Kavanaugh (2000)	28	5, 10, 20	40	[16]
Charles (2000)	193	1, 3, 10	14	[39]
Quinn (2001)	20	3	14	[36]
Taylor (2001)	24	5	18	[40]
Shergy (2002)	553	3	14	[30]
Total	1546			

Table 2. Controlled studies in rheumatoid arthritis using infliximab – secondary areas of interest.

Area of interest	Citation (ATTRACT: all subanalyses)	n	Dosage (mg/kg)	Duration (weeks)	Ref.
Radiologic comparison	Lipsky ATTRACT (1999)	428	3, 10	30	[31]
	Van der Heijde ATTRACT (2001)	428	3, 10	30	[32]
	Antoni ATTRACT (2001)	428	3, 10	30	[33]
	Maini ATTRACT (2001)	428	3, 10	30	[34]
	Wong ATTRACT (2002)	428	3, 10	30	[35]
Pharmacokinetics	Maini (1998)	101	1, 3, 10	26	[18]
	St Clair ATTRACT (2001)	428	3, 10	30	[37]
Immunogenicity	Lorenz (1996), Lorenz (2000)	18	1, 10	4	[38,42]
	Paleolog (1996)	73	1, 10	4	[23]
	Tak (1996)	14	10, 20	4	[24]
	Maini (1998)	101	1, 3, 10	26	[18]
	Charles (2000)	193	1, 3, 10	14	[39]
	Taylor (2001)	24	5	18	[40]
Time to onset	Emery ATTRACT (2001)	428	3, 10	30	[41]
	Shergy (2002)	553	3	14	[30]
Safety of multiple infusions	Lorenz (2000)	18	1, 10	4	[42]
	Maini (1998)	101	1, 3, 10	26	[18]
	Charles (2000)	193	1, 3, 10	14	[39]
Rate of infusion	Isern (2001)	553	3	14	[43]
	Shergy (2002)	553	3	14	[30]
Quality of life	Kavanaugh ATTRACT (2001)	428	3, 10	30	[44]
	Kavanaugh ATTRACT (2002)	428	3, 10	30	[45]
Steroid tapering	Shergy ATTRACT (2002)	553	3	14	[30]

ATTRACT: Anti-TNF Trial in Rheumatoid Arthritis with Concomitant Therapy.

Table 3. Uncontrolled studies in rheumatoid arthritis using infliximab – secondary areas of interest.*

Area of interest	Citation	n	Ref.
Favourable results in juvenile RA	Kimura (2001)	4	[47]
	Chaturvedi (2002)	9	[48]
	Masatlioglu (2002)	12	[49]
	Honaken (2002)	26	[50]
Successful tapering of concomitant therapies to evaluate infliximab monotherapy	Schimizzi (2001)	156	[51]
Efficacy and safety of infliximab and leflunomide	Kiely (2002)	20	[52]
Efficacy and benefits of infliximab and azathioprine	Durez (2002)	21	[53]
Efficacy and safety of infliximab among patients with inadequate response to etanercept	Shergy (2002)	40	[54]
Behavior of chemokines and the involvement of IL-8, MCP-1	Taylor (1999)	10	[55]
Behavior of chemokines and the involvement of IL-1α and IL-1β	Ulfgren (2000)	8	[56]
Confirmation of ACR20 results at 50 sites (Germany)	Antoni (2001)	263	[57]
Confirmation of ACR20 results (Belgium)	Durez (2002)	133	[53]
Confirmation of ACR20 results (Canada)	Maksymowych (2001)	95	[60]
Confirmation of ACR20 results – registry	Wolfe (2002)	5028	[61]
Neutral effect on pregnancy	Antoni (2001)	59	[58]
Favourable response to monotherapy using HAQ evaluations	Erkan (2001)	27	[59]
Comparison of etanercept with respect to efficacy and safety in patients with moderate-to severe RA	Ridley (2002)	157	[66]
Early RA	Quinn (2002)	20	[63,64]
	Taylor (2002)	24	[65]
Unemployment	Van Vollenhoven (2002)	296	[62]

ACR: American College of Rheumatology response criteria; ATTRACT: Anti-TNF Trial in Rheumatoid Arthritis with Concomitant Therapy; HAQ: Health assessment questionnaire; MCP: Monocyte chemoattractant protein; RA: Rheumatoid arthritis.

*Includes case studies, prospective studies, retrospective studies, episodic treatments, cohorts, varying dosages and durations.

Table 4. Controlled studies in Crohn's disease using infliximab – primary endpoint efficacy.

Citation	n	Dosage (mg/kg)	Duration (weeks)	Ref.
Van Dullemen (1995)	10	5, 10	8	[67]
McCabe (1996)	20	1, 5, 10, 20	12	[68]
Targan (1997)	108	5, 10, 20	12	[69]
Present (1999)	94	5, 10	6	[70]
Hanauer (2002)	573	5, 10	54	[71]
Sands (2002)	306	5	54	[72]

Table 5. Controlled studies in Crohn's disease using infliximab – secondary areas of interest.

Area of interest	Citation	n	Dosage (mg/kg)	Duration (weeks)	Ref.
Ability to sustain remission	Rutgeerts (1999)	73	10	32	[73]
Affect on endoscopic healing	D'Haens (1999)	30	5, 10, 20	4	[74]
Affect on downregulation of oleocolitis	Baert (1999)	13	5, 10, 20	4	[75]
Successful fistula closing	Present (1999)	94	5, 10	6	[70]
ACCENT I					
Mucosal healing in refractory CD	Rutgeerts (2002)	573	5, 10	54	[76]
Preference for scheduled over episodic treatments	Rutgeerts (2002)	573	5, 10	54	[77]
Mucosal healing	Rutgeerts (2002)	573	5, 10	54	[76]
Preference for induction regimen over single dose	Fasanmade (2002)	573	5, 10	54	[78]
Reduction or discontinuation of concomitant steroids	Lichtenstein (2002)	573	5, 10	54	[79]
Improvement in QOL; patients returning to work	Lichtenstein (2002)	573	5, 10	54	[80]
	Lichtenstein (2002)	573	5, 10	54	[83]
	Feagan (2002)	573	5, 10	54	[81]
	Feagan (2002)	573	5, 10	54	[82]
No increase in intestinal strictures	Lichtenstein (2002)	573	5, 10	54	[103]
Positive economic impact to healthcare system	Colombel (2002)	573	5, 10	54	[85]
	Rutgeerts (2002)	573	5, 10	54	[76]
Determination of serum markers as early warning of disease	Esters (2002)	573	5, 10	54	[15]
	Geboes (2002)	573	5, 10	54	[88]
Determination of CRP changes as early warning of disease	Sands (2002)	573	5, 10	54	[89]
Sustained remission lowers hospitalisation and surgery	Lichtenstein (2002)	573	5, 10	54	[86]
ACCENT II					
Reduced hospitalisations	Lichtenstein (2002)	282	5	54	[87]
Improved disability and unemployment	Feagan (2002)	306	5	54	[84]

ACCENT: A Crohn's Disease Clinical Trial Evaluating Infliximab in a New Long-Term Treatment Regimen; CD: Crohn's disease; CRP: C-reactive protein; QOL: Quality of life.

Table 6. Uncontrolled studies in Crohn's disease using infliximab – secondary areas of interest.*

Area of interest	Citation	n	Ref.
Corroboration of results of clinical trials	Cohen (US) (2000)	129	[90]
	Arnott (UK) (2001)	50	[91]
	Asakura (Japan) (2001)	25	[92]
	Farrell (US) (2000)	100	[95]
	Mortimore (Australia) (2001)	57	[96]
	Hommes (The Netherlands) (2002)	73	[97]
	Kennedy (UK) (2002)	19	[98]
	Sample (Canada) (2002)	109	[99]
	Glazier (US) (2002)	83	[100]
Efficacy of thalidiomide for maintenance after infliximab induction	Sabate (2002)	15	[93]
Increased apoptosis of inflammatory cells causing lamina propia	Ten Hove (2002)	10	[94]
Remission of ileoanal pouch	Ricart (2001)	7	[101]
Healing of lesions in perineal cutaneous CD	Geyer (2000)	1	[102]
Granuloma annulare	Fleisher (2002)	1	[107]
CD patients with HIV	Tessnow (2002)	3	[108]
Reduction of strictures	Weinberg (2002)	127	[104]
Refractory pouchitis	Lodhavia (2002)	1	[105]
Extraintestinal manifestations	Parsi (2002)	81	[106]
Decrease in healthcare resources	Rubenstein (2002)	79	[119]

CD: Crohn's disease.
*Includes case studies, prospective studies, retrospective studies, episodic treatments, cohorts, varying dosages and durations.

Table 7. Uncontrolled studies in Crohn's disease using infliximab – children.*

Area of interest	Citation	n	Ref.
Pharmacokinetics in children	Hadigan (1999)	21	[109]
	Fasanmade (2002)	62	[110]
Steroid tapering in children	Vanderhoof (2002)	1	[111]
General improvement and remission in children	Vasiliasuskas (1999)	17	[112]
	Hyams (2000)	19	[113]
	Escher (2002)	1	[114]
	Mamula (2002)	9	[115]
Episodic treatments in children	Kugathasan (2002)	86	[116]
In-office infusions in children	Cohen (2002)	43	[117]
QOL in children	Serrano (2001)	18	[118]

QOL: Quality of life.
*Includes case studies, prospective studies, retrospective studies, episodic treatments, cohorts, varying dosages and durations.

Table 8. Controlled and uncontrolled studies in ankylosing spondylitis using infliximab.

Citation	n	Dosage (mg/kg)	Ref.
Controlled studies			
Braun (2002)	70	5	[137,139]
Van den Bosch (2002)	40	5	[138]
Uncontrolled studies			
Brandt (2000)	11	5	[135]
Van den Bosch (2000)	21	5	[136]
Baeten (2001)	21	5	[143]
Boeger (2001)	1	5	[150]
Breban (2001)	50	5	[144]
Kaiser (2001)	2	3	[151]
Stone (2001)	21	5	[145]
D'Agostino (2002)	2	3	[152]
Hadi (2002)	1	5	[153]
Kruithof (2002)	21	5	[146]
Maksymowic (2002)	21	3	[147]
Sidiropoulos (2002)	16	4	[148]
Boki (2002)	15	3	[149]

Table 9. Controlled and uncontrolled studies in psoriasis and psoriatic arthritis using infliximab.

Citation	Indication	n	Dosage (mg/kg)*	Ref.
Controlled studies				
Chaudhari (2001)	Ps	33	5, 10	[171]
Antoni (2002)	Ps	102	5	[174]
Uncontrolled studies				
Prospective				
Ogilvie (2001)	Ps	6	5	[176]
Schopf (2002)	PsA, Ps	8	5	[177]
Cherouvim (2002)	PsA	12	3	[178]
Feletar (2002)	PsA, Ps	16	5	[179]
Retrospective				
Bolce-Stevens (2001)	PsA	7	Average 3.5	[180]
Stevens (2002)	PsA, Ps	29	3 – 7	[181]
Fleischmann (2001)	PsA, Ps	31	Average 3.6	[182]
Drosou (2002)	Ps	6	5	[183]
Case studies				
Kirby (2001)	Ps	1	5	[184]
Ruzicka (2001)	Ps	1	3	[185]
Tan (2001)	Ps	1	5	[186]
Bray (2001)	PsA	3	3	[188]
Mang (2002)	Ps, PsA	1	3	[189]
Menter (2002)	Ps	1	200 mg	[190]
O'Quinn (2002)	Ps, PsA	2	5, 10	[187]

Ps: Psoriasis; PsA: Psoriatic arthritis.
*Unless otherwise specified.

Table 10. Studies in other indications using infliximab.

Area of interest	n	Citation	Ref.
Amyloid A amyloïdosis	12	Gottenberg (2002)	[218]
	6	Ortiz-Santamarnia (2002)	[219]
Biliary cirrhosis	1	Fleichtner (2001)	[220]
Celiac disease	1	Gillett (2002)	[221]
Erythema nodosum	1	Fiske (2002)	[222]
Giant cell arteritis	4	Cantini (2001)	[223]
	1	Airó (2002)	[224]
Graft-versus-host disease	1	Boh (2002)	[225]
	4	Rivkina (2002)	[226]
Hidradenitis supporativa	5	Sullivan (2002)	[227]
Kawasaki's disease	1	Weiss (2002)	[228]
Mixed connective tissue/lupus	4	Scott (2002)	[229]
Panniculitis	1	Humeniuk (2002)	[230]
Polyarthritis	3	Wouters (2002)	[232]
	19	Nossent (2001)	[231]
Polychondritis	1	Ehresmann (2002)	[233]
Refractory mononeuritis	1	Garcia-Porrua (2002)	[234]
Reiter's syndrome	1	Gaylis (2002)	[235]
Sacroiliitis	5	Kellner (2002)	[236]
Sarcoidosis	3	Baughman (2001)	[237]
	1	Yee (2001)	[238]
	6	Fahey (2002)	[239]
	3	Sweiss (2002)	[240]
Sjögren's syndrome	16	Steinfeld (2001)	[241]
Still's disease	6	Kraetsch (2001)	[242]
	2	Hufstutter (2002)	[243]
Wegener's granulomatosis	6	Lamprecht (2002)	[244]
Early onset sarcoid arthritis	3	Brescia (2002)	[245]
Vascular endothelial dysfunction	10	Forster (2002)	[246]
Recalcitrant subcorneal pustular dermatosis	1	Voigtländer (2001)	[247]